PCRD Nucleic Acid Detector

Intended Use

PCRD Nucleic Acid Detector is a single–use *in vitro* immunochromatographic test for the qualitative visual detection of labelled PCR amplicons.

Intended End User

PCRD Nucleic Acid Detector is intended for research use only.

Background

Traditionally, the outcome of PCR assays has been visualised using time-consuming methods such as agarose gel electrophoresis. However, this requires specialised equipment and toxic chemicals.

PCRD lateral flow test strips are a rapid, safe and sensitive way to confirm the successful outcome of DNA amplification. The strips incorporate reagents which sequentially capture and allow visualisation of double-stranded amplification products containing selected binding partners. This simple method of amplicon detection is complete in 10 minutes, and the result is detectable by the naked eye due to an aggregation of carbon particles at the capture line. As an alternative to ethidium bromide staining of agarose gels, PCRD offers greater speed, safety, sensitivity and decreased labour.

The lateral flow test strip is generic, and can be used to detect the amplification product of any reaction in which appropriately labelled primers are included. The strip can be used with nucleic acid amplification methods such as PCR, LAMP, RPA and HDA.

Principle

The PCRD Nucleic Acid Detector is a sandwich immunochromatographic assay (Figure 1) based upon lateral flow technology. The lateral flow assay has three reaction lines:

1) Line 1, closest to the sample application port, detects DIG/Biotin-labelled amplicons.

2) Line 2 detects FAM/Biotin- or FITC/Biotin-labelled* amplicons.

**N.B. Although this line will detect both Fluorescein-containing fluorophores (FITC and FAM), for clarity it will henceforth be referred to as the "FITC/Biotin" line.*

3) Line 3 or C-line, furthest away from the sample port, which is a flow-check control line.

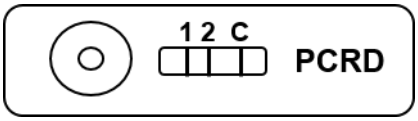


Figure 1. Layout of a typical PCRD device showing three lines.

Upon sample application, the Neutravidin coated carbon particles are rehydrated and react with the tag/Biotin-labelled amplicon in the sample. The mixture travels along the membrane by capillary action. As the sample flows through the test membrane, the coated particles migrate and, in the presence of the FITC/Biotin and DIG/Biotin-labelled amplicons, are captured by the antibodies immobilized at the test lines (T-lines) to form a coloured complex, and therefore a visible line.

The strip contains a control line so that negative results are indicated by the presence of a single black line on the test strip (C-line). Positive results are recognised by the presence of black lines on the strip at the

T-line positions. The test is read after 10 minutes, however results may start to appear before this.

Primer Design

PCRD Nucleic Acid Detector requires only simple 5′- modifications of your normal primer pair: Biotin, plus one from DIG or FITC on the alternate primer, allowing for reactions to be multiplexed if desired. The order of incorporation of each modification onto the forward or reverse primer has been demonstrated not to influence the efficacy of incorporation of the tags into the amplicon; however some optimisation may be necessary.

Precautions

The reagents are not considered dangerous according to the 2012/18/EU and 1272/2008 directives. However, they should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

- Keep PCRD devices in a horizontal position while the sample is loaded.
- Do not use components past their expiration date.
- Do not mix components from different kit lots.
- Handle all test specimens with care to avoid infection with potentially hazardous fluids.
- Avoid skin contact by wearing protective disposable gloves.
- Sodium azide (NaN3) contained in some of the kit reagents may react with lead or copper plumbing and form highly explosive metal azides (Please refer to local regulations for the disposal of sodium azide).
- When disposing of large volumes of reagents flush with copious amounts of water to avoid a build-up.
- The test should be discarded in an appropriate biohazard container after use.
- The test cartridge is for single use only. Do not re-use.
- Devices which show a discoloured membrane following sample application should be discarded and a new sample provided for analysis.

Storage and Stability

The kit must be stored in its original sealed packaging between 10°C and 25°C. The test and reagents are stable until the expiration date printed on the packaging. Do not use the kit beyond its labelled expiration date. Only remove the device from the foil packaging immediately before use.

Kit Contents

- Test device (×50)
- PCRD Extraction Buffer bottle (×5)
- Instructions for use (this document).

Materials required but not provided

- Mixing tubes.
- Pipettes and tips.

Recommendations

The recommendations in this guide are based on a variety of amplification methods and running times. Since amplification methods vary in their efficiencies, it is recommended that a series of dilutions are carried out when using the strips for the first time with a new amplification method. The intensity of one or both of the T-lines (Line 1 or Line 2) will be a function of the efficiency of your PCR reaction, the duration of amplification and the level of dilution with buffer. Some optimisation may be necessary to determine the reaction conditions which give rise to clearly visible T-lines. T-line intensities may vary; even faint lines should be treated as positive.

Primary Sample Collection, Handling and Storage Assay Procedure

The example below is given for illustration purposes only. Run volume should be 75µl.

The dilution and amount of your amplicon should be optimised for each PCR target.

- Carry out a nucleic acid amplification reaction according to your usual protocol.
- Pipette 6µl of the amplification product into a 0.5ml tube.
- Transfer 84µl of the PCRD Extraction Buffer supplied into the tube and mix thoroughly.
- Open the foiled device immediately before use. Do not use a PCRD lateral flow test strip which has been left outside the foil pouch unused for an extended period of time. Remove the PCRD test strip and place onto a clean, level surface.
- Add 75µl of the diluted reaction mixture to the sample well of a PCRD test cassette.
- Leave the cassette in a horizontal position for 10 minutes while the result develops.
- Read the result visually at 10 minutes. Ignore any changes which occur after 10 minutes.

Interpretation of Results

This assay detects nucleic acid created during nucleic acid amplification reactions. The efficiency of the reaction and concentration of your starting material may affect amplicon yield. Therefore, we advise that preliminary tests are carried out to determine the concentration of your sample.

Tests on which the control line does not appear should be deemed invalid and a fresh test should be run. Examples of invalid tests are shown in Figure 2. The absence of a C line may be due to the presence of high levels of amplicon in the sample and further dilutions may need to be performed prior to running the PCRD test.

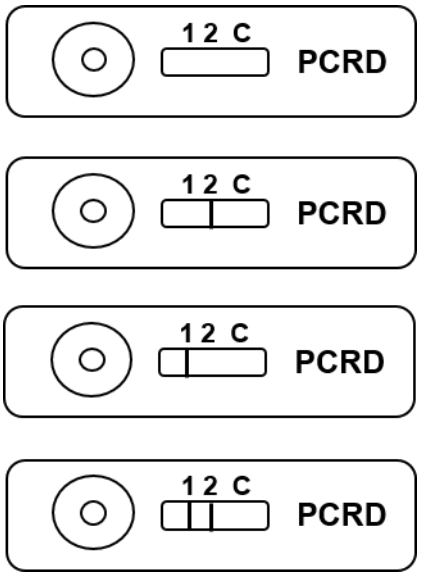
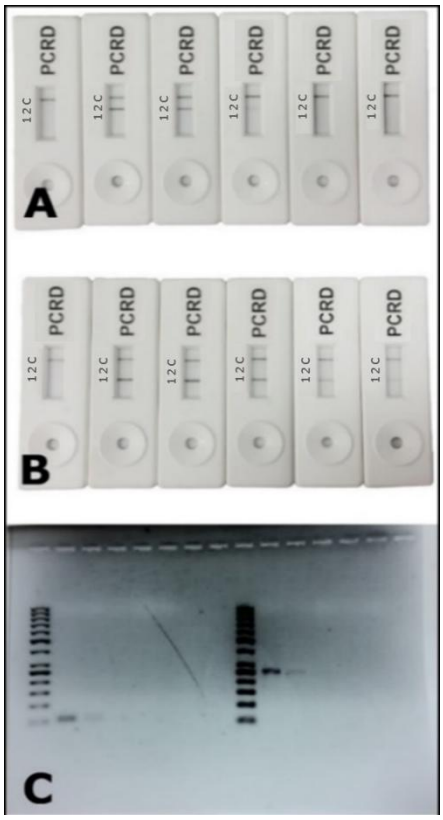


Figure 2. Examples of potential outcomes where a test should be deemed invalid.

Figure 3 shows the results of a PCR amplification using DNA from *Solanum tuberosum* (potato) with primers for 18s modified with Biotin/FAM or COX primers modified with Biotin/DIG. The amplicon was measured using a Nanodrop 1000 spectrometer and was found to contain 10µg/ml of dsDNA. Samples were diluted in nuclease free water from zero dilution to 1 in 1000. Equivalent volumes of samples were run on either a 2% agarose gel (Figure 3C) or further diluted with Extraction Buffer and run on the PCRD device (Figure 3A = FITC, Figure 3B = DIG). Results are shown for comparison above.



FITC DIG

Figure 3. A typical concentration curve achieved by the dilution of PCR amplicons compared with the same samples run on a 2% agarose gel

Troubleshooting

Sample Migration Does Not Start or is Not Homogeneous/Control Line Does Not Appear or is Weak

- Device or membrane is damaged: repeat the assay with a new device.
- Sample volume is not adequate: repeat the test using the correct amount of sample (75µl)
- Keep the LFD in the foil pouch for as long as possible to reduce the risk of cross contamination
- Ensure your primers and templates are compatible for use with PCRD. Read primer design section
- For customers who have previously used PCRD-2- PCRD is more sensitive and this should be considered
- Ensure the steps highlighted in the Primary Sample Collection, Handling and Storage Assay Procedure section are followed

Limitations

This assay is for the detection of PCR amplicons doubly labelled with DIG/Biotin and FITC/Biotin only. Unlabelled or singly labelled PCR amplicons will not be detected by this assay.

Performance Characteristics

Assay Range: For guidance, in lab studies PCRD has been shown to detect between 0.02µg/ml and 0.001µg/ml dsDNA. However, levels of sensitivity will depend on the outcome of your own PCR. The detection level for your assay should therefore be determined empirically.

Specificity: PCRD is specific for the detection of DIG and FITC. It does not detect Dinitrophenol (DNP) or Sulforhodamine 101 Acid Chloride (Texas Red).

Considerations

- An initial quality control check on your primer pair and DNA is advised. PCRD is highly sensitive and may detect the presence of primer dimers and non-specific product as a “positive”.
- Keep the LFD in foil pouch for as long as possible to reduce the risk of cross contamination.
- Ensure the steps highlighted in the Primary Sample Collection, Handling and Storage Assay Procedure section are followed.

References

The references below are provided as examples of the use of lateral flow devices for detection of PCR amplicons.

Tomlinson, J. A., Dickinson, M. J., Boonham, N., (2010) "Rapid detection of Phytophthora ramorum and Phytophthora kernoviae by two minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device", *Phytopathology* **100**(2): 143-149.

James H E, Ebert K, McGoingle R, Reid S M, Boonham N, Tomlinson J A, Huthings G H, Denyer M, Oura C A L, Dukes J P, and King D P (2010) "Detection of African Swine fever virus by loop-mediated isothermal amplification (LAMP)"., *Journal of Virological Methods,* **164:** 68-74.

Kartin Krõlov, Jekayerina Frovlova, Oana Tudoran, Julia Suhorutsenko, Taavi Lehto, Hiljar Sibul, Imre Mäger, Made Laanpere, Indrek Tulp, Ülo Langel, (2014) "Sensitive and Rapid Detection of Chlamydia trachomatis by Recombinase Polymerase Amplification Directly from Urine"., *Journal of Molecular Diagnostics* (Vol. **16**, No. 1 January 2014).

Pastey, M., (2014) "Point-of-care detection of Tritrichomonas Foetus in 30 minutes using Recombinase Polymerase Amplification (RPA) technology and lateral flow device (LFD) as a novel diagnostic tool in clinical settings". Oregon State University, College of Veterinary Medicine.

Technical Support

For technical support concerning the PCRD Nucleic Acid Detector please contact a member of our team on +44 (0) 1904 406050 or info@abingdonhealth.com. Unfortunately we are unable to offer technical support concerning your amplification reaction method or design.

Manufacturer

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Symbols Used

	Do Not Reuse		Expiry Date
	Catalogue Number		Instructions for Use
	Contains Sufficient for <n> Tests		Temperature Limits
	Lot Number		Manufacturer