

## ViroInspect™ Rodent 1

Test Kit for the Detection and Identification of Rodent Parvoviruses, Porcine Circoviruses 1 and 2 and Vesivirus 2117 in Biologics

# ViroInspect™ Rodent 1

## A Fast and Easy-to-Use Virus Detection Assay

A key requirement for the production of biopharmaceutical products is to ensure the absence of any adventitious agents, including viruses.

In addition to the impact on product safety, contamination events can cause the loss of product, loss of production time, loss of reputation and considerable drawbacks in terms of costs. Several cell culture manufacturing companies have been negatively affected by a viral contamination in the last 20 years.

Industrial knowledge about contaminating adventitious viruses and successful approaches to counteract a contamination event is limited to each company's individual experience. Contemporarily, cell culture and in vivo susceptibility test are still used as standard methods but suffer from many disadvantages such as time, sample throughput, serviceability, specificity, sensitivity, ethical aspects and overall costs. At that point the technology of Greiner Bio-One becomes an innovative solution.

### ViroInspect™

ViroInspect™ is characterised by short throughput times, a high sensitivity, specificity, robustness, repeatability and intermediate precision. It provides fast data analysis and the delivery of simple and precise results that enable 'go' and 'no-go' decisions in a timely manner.

ViroInspect™ is combining multiplexing polymerase chain reaction (PCR) and microarray technology. It is part of a complete assay system consisting of the ViroInspect™, the CheckScanner™ and the CheckReport™ Software. The ViroInspect™ kit itself provides integrated sample preparation, extraction of viral DNA and RNA, reverse transcription, PCR amplification, and microarray hybridisation.

ViroInspect™ is based on a modular system.

### ViroInspect™ Rodent 1

The first modul, ViroInspect™ Rodent 1, is a ready-to-use, qualitative test kit for the detection and identification of Rodent Parvoviruses<sup>1</sup> as displayed in Table 1 (including Hamster Parvovirus, Mouse Minute Virus, Mouse Parvovirus, Kilham Rat Virus, Toolan's H1 Virus, Rat Parvovirus, Rat Minute Virus), Porcine Circoviruses 1 and 2, Vesivirus 2117 and related isolates in biological

materials such as cell culture samples. Thus, the ViroInspect™ Rodent 1 focuses on (1) viruses from which is known that rodents are their natural hosts, (2) viruses which infect CHO cells or rodent cells and induce a productive infection with or without triggering a cytopathic effect, and (3) viruses which have been detected before in CHO cultures as well as in bioreactors, respectively.

The identification of the adventitious viruses mentioned before allows a comprehensive root-cause-analysis and an immediate corrective action in case of a contamination event that is caused by one of these viruses.

With results delivered in less than 9 hours, ViroInspect™ Rodent 1 is a rapid and effective alternative to cell culture and in vivo testing. Day to day flexibility as well as a high throughput of samples are assured as the system can handle up to 48 samples in parallel.

#### ViroInspect™ Rodent 1 at a Glance

- Detection and identification of Rodent Parvoviruses (including Mouse Minute Virus), Porcine Circoviruses 1 and 2, Vesivirus 2117 and related isolates allow a thorough root-cause-analysis
- Time-to-results in less than 9 hours
- Microarray-based detection system
- Integrated sample preparation, extraction of viral DNA and RNA, reverse transcription, PCR amplification, microarray hybridisation, and software-based evaluation
- Comprehensive on-chip controls
- Processing of sample volumes of up to 10 mL
- Validated in compliance with the ICH Q2(R1) guideline
- Rapid, automated digital result analysis and report generation
- CheckReport™ Software developed acc. to FDA electronic records regulations (21 CFR part 11)

<sup>1</sup> The term Rodent Parvoviruses is used as synonym for viruses detected with ViroInspect™ Rodent 1 that belongs to the Family Parvoviridae, Genus Protoparvovirus, Species Rodent Protoparvovirus 1 and 2 and unclassified Protoparvoviruses.

# Working Schedule for the VirolInspect™ Rodent 1

## Virus Detection and Identification within 9 hours



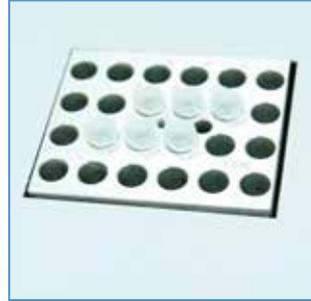
### 1 Sample preparation

Duration: 90 minutes



### 2 DNA/RNA extraction

Duration: 60 minutes



### 3 Reverse transcription

Duration: 70 minutes



### 4 Purification

Duration: 25 minutes



### 5 PCR

Duration: 210 minutes



### 6 Hybridisation

Duration: 40 minutes



### 7 Washing & Drying

Duration: 15 minutes



### 8 Scanning & Analysis

Duration: 15 minutes

## Assay Principle

The VirolInspect™ Rodent 1 workflow starts with one freeze and thaw cycle for breaking up rodent cells, followed by the concentration of the cell lysate using a centrifugal filter device after clearing the supernatant from cell debris by centrifugation.

After sample preparation, viral DNA and viral RNA are extracted and purified. The viral RNA is transcribed into cDNA by specific and highly conserved primers. The cDNA product is purified followed by PCR amplification of viral DNA and viral cDNA molecules in the presence of specific and highly conserved primers.

dUTP is incorporated into the ready-to-use VirolInspect™ PCR MasterMix. Thus, treatment with Uracil-N-Glycosylase (UNG) eliminates the risk of carry-over contaminations from previous PCR reactions. Labelled PCR products are then hybridised to complementary DNA probes present in five replicates on the microarray. Subsequent washing steps remove unbound amplification products. The VirolInspect™ Rodent 1 chip is scanned, analysed and evaluated using the CheckScanner™ and CheckReport™ Software. A report is created that indicates the presence or absence of the adventitious viruses, respectively.

Table 1: Viruses detectable by the VirolInspect™ Rodent 1

Family	Genus	Species/Viruses
Parvoviridae	Protoparvovirus	Mouse Minute Virus (MVM)
		Hamster Parvovirus (HaPV)
		Mouse Parvovirus (MPV)
		Kilham Rat Virus (KRV)
		Toolan's H1 Virus (H1)
		Rat Parvovirus (RPV)
		Rat Minute Virus (RMV)
Calciviridae	Vesivirus	Vesivirus 2117 and related isolates
Circoviridae	Circovirus	Porcine Circovirus 1 (PCV1)
		Porcine Circovirus 2 (PCV 2)



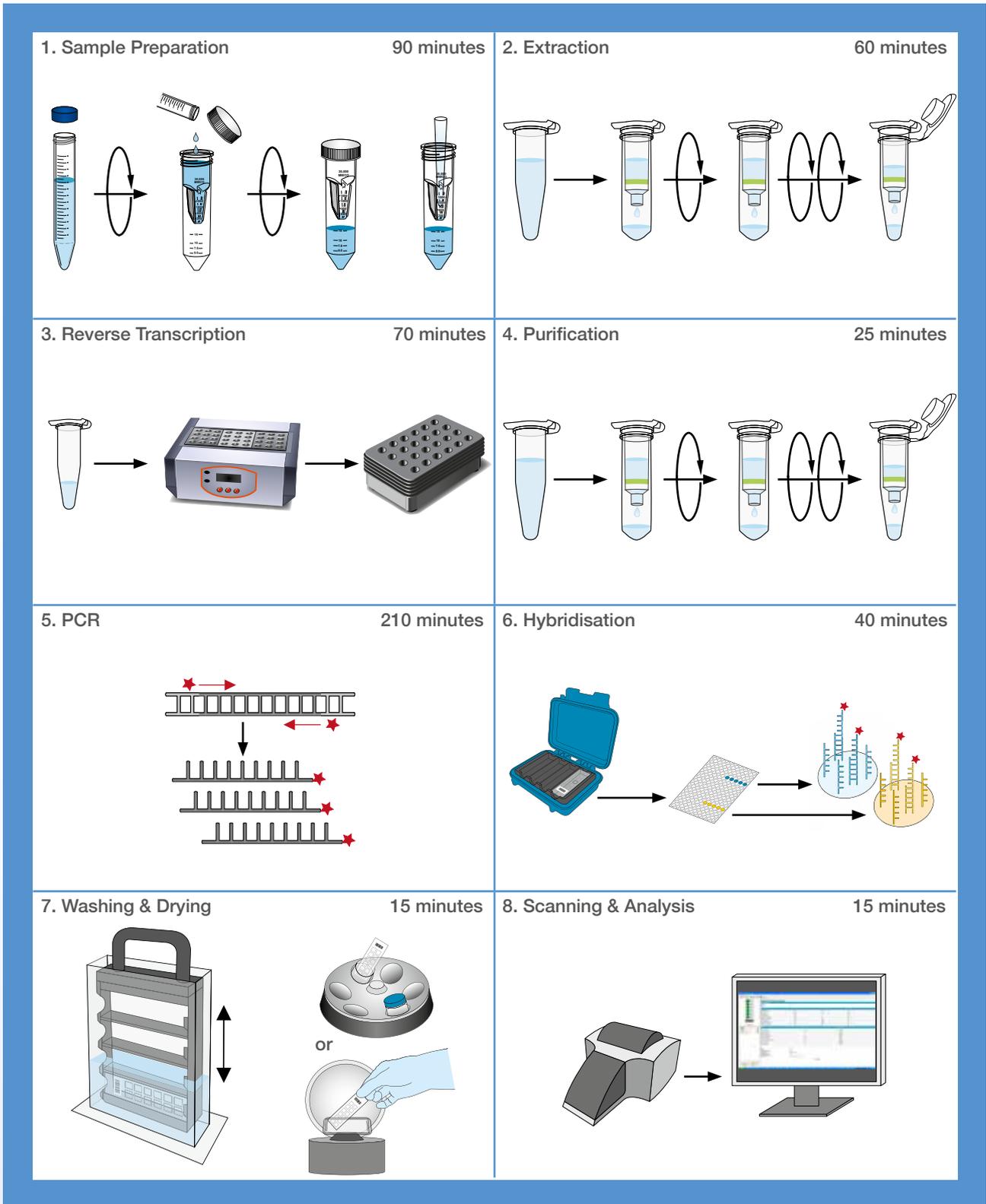


Figure 1: Detailed Overview of the Virolnspect™ Rodent 1 assay procedure

## ViroInspect™ Rodent 1 Chip Design and On-Chip Controls

The ViroInspect™ Rodent 1 chip is based on the well-established Greiner Bio-One HTA™Slide<sup>2</sup> that allows the parallel analysis of 6 and 12 samples, respectively. The chip has either 6 or 12 compartments containing one microarray.

Each microarray is comprised of 19 different probes spotted in five replicates. 14 viral specific probes enable the detection of the viruses listed in Table 1 (red channel: 635 nm). In case of Rodent Parvoviruses, a universal probe allows the combined detection of all Parvoviruses that can be identified by ViroInspect™ Rodent 1.

All requested controls, as described in Ph. Eur. Monograph 2.6.21, have been implemented into the system. Thus, five controls and a printing control for all spots enable the monitoring of the assay performance such as the specimen quality, the performance of DNA and RNA extraction, the reverse transcription, the PCR reaction (red channel: 635 nm) as well as the presence / homogeneity of each individual DNA spot and the hybridisation efficiency (green channel: 532 nm).

<sup>2</sup>The HTA™Slide platform is covered by U.S. Patent No. 8.007.744 .

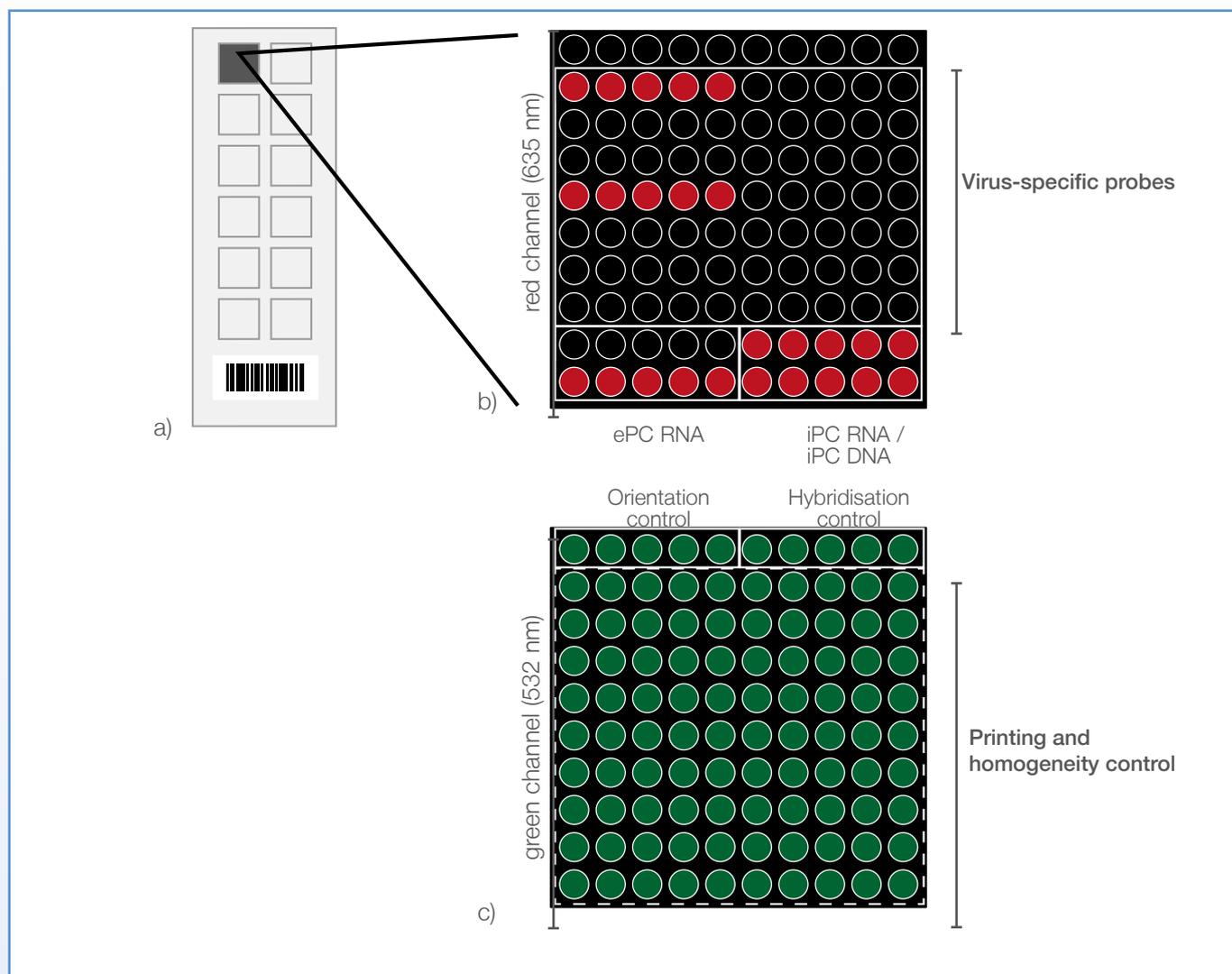


Figure 2: Design of the ViroInspect™ Rodent 1 chip

a) Schematic drawing of the ViroInspect™ Rodent 1 chip with 6 or 12 independent compartments containing one microarray.  
 b) and c) Microarray images displayed by the CheckReport™Software for the two different excitation wavelengths used for scanning (b) red channel: 635 nm; c) green channel: 532 nm) and schematic drawings of the ViroInspect™ Rodent 1 chip layout. Virus-specific probes and on-chip controls are indicated.





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