

High Speed Biopharmaceutical Quality Control

ViroInspect[®] Rodent 1 and ViroInspect[®] Rodent 2 for the Detection and Identification of 21 Contaminating Viruses

ViroInspect® Rodent 1 and Rodent 2

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® kits themselves provide 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 and Rodent 2

The first module, ViroInspect® Rodent 1, is a ready-to-use, qualitative test kit for the detection and identification of Rodent Parvoviruses¹ 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. The second module, the ViroInspect® Rodent 2, enables qualitative detection and identification of Rodent Calici-, Corona-, Paramyxo-, Picorna- and Reoviruses in the same samples types.

Thus, the ViroInspect® test kits focus 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® 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® at a Glance

- Detection and identification of 10, 11 or up to 21 contaminating adventitious viruses 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)

¹ 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 ViroInspect® Rodent 1 and Rodent 2

Virus Detection and Identification within 9 hours



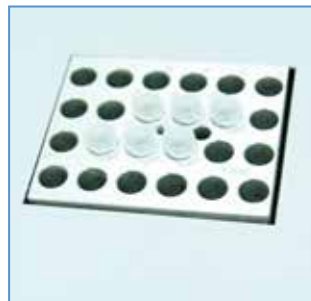
1 Sample collection

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

Detectable Viruses

Table 1: Viruses detectable by the Virolnspect® Rodent 1

Family	Genus	Species/Viruses
Parvo- viridae	Protoparvo- virus	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)

Table 2: Viruses detectable by the Virolnspect® Rodent 2

Family	Genus	Species/Viruses
Calci- viridae	Norovirus	Murine Norovirus (MNV)
Corona- viridae	Beta-Coro- navirus	Mouse Hepatitis Virus (MHV)
		Rat Coronavirus (RCV)
		Sialodacryoadenitis Virus (SDAV) =Rat Coronavirus
Paramyxo- viridae	Pneumo- virus	Pneumonia Virus of Mice (PVM)
	Rubulavirus	Parainfluenza virus 5 (PIV5), formerly known as simian virus 5 (SV5)
	Respirovirus	Sendai Virus (SeV)
Picorna- viridae	Cardiovirus	Encephalomyocarditis Virus (EMCV)
		Theiler's Murine Encephalo- myelitis Virus (TMEV)
Reoviridae	Rotavirus	Murine Rotavirus (EDIM = Epizootic Diarrhea of Infant Mice)
	Orthoreo- virus	Mammalian Orthoreovirus 1-3 (MRV 1-3)

Assay Principle

The Virolnspect® 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 Virolnspect® 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 Virolnspect® 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.

ViroInspect® Chip Design and On-Chip Controls

The ViroInspect® chips are based on the well-established Greiner Bio-One HTA™Slide² that allows the parallel analysis of 6 samples. The chips have 12 independent compartments, of which six contain a microarray. Each microarray is comprised of different probes spotted in five replicates. Virus specific probes enable the detection of the viruses listed in Table 1 and 2, respectively (red channel: 635 nm). This also includes some group-specific probes that detect a specific panel of viruses or a specific virus and its close relatives.

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).

²The HTA™Slide platform is covered by U.S. Patent No. 8.007.744 .

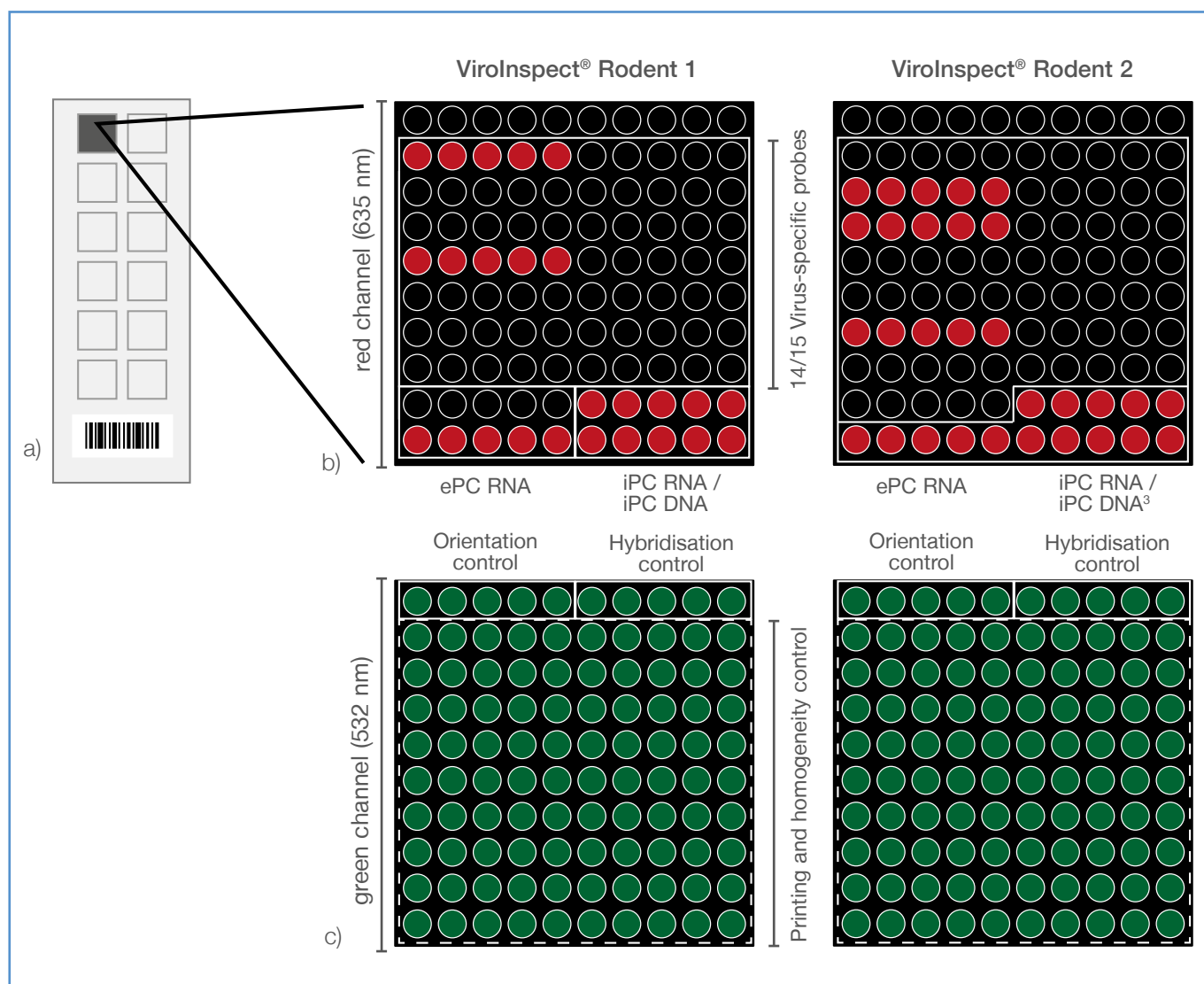


Figure 1: Design of the ViroInspect® Rodent 1 and Rodent 2 chip

a) Schematic drawing of the ViroInspect® chips with 12 independent compartments, of which six contain a 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® chip layouts virus-specific probes and on-chip controls are indicated.

³In case of ViroInspect® Rodent 2, the internal Positive Control DNA (iPC DNA) is not evaluated by the CheckReport Software, hence no DNA viruses are analysed with this ViroInspect® module. Nevertheless, the addition of the internal Positive Control DNA to the analysis is necessary for monitoring the whole analysis process, e.g. in cases of technical support or if the eluates are analysed with other ViroInspect® modules.

Automated Analysis by the CheckScanner and the FDA Compliant CheckReport Software

Upon hybridisation and washing the automated analysis of the chip is performed by use of the CheckScanner and the CheckReport Software. The presence / absence of adventitious viruses and the on-chip controls are automatically evaluated and a report is generated (Figure 2). The CheckReport Software is in accordance to the FDA regulations on electronic records (21 CFR part 11) and produces QM-compliant electronic documentation.

Moreover, the CheckScanner – in combination with the CheckReport Software – can be integrated into a laboratory information management system (LIMS).

Control	Channel	Value
Administration Control	002	013.2
Authentication Control	008	008.6
Authorization Control	002	0
Creation Control	002	047.8
Deletion Control	008	0.0
External Filesystem Control	002	0.0
Internal Filesystem Control	002	0.0
Internal Filesystem Control	008	0.0

Figure 2: Report generated by the CheckReport Software results.

The CheckReport Software is easy to use by an intuitive software interface. Thereby, ViroInspect® derived results are quickly obtained and provide a comprehensive understanding. Barcodes enable the traceability of the chips.

ViroInspect® Performance Data

Result Type	qualitative virus detection and identification
Time to Result	~9 hours
Sample volumes	up to 10 mL
PCR specifications	multiplex PCR, ready-to-use MasterMix containing dUTP
Limit of Detection	ViroInspect® Rodent 1: 3-8 IFU/reaction ⁴ ViroInspect® Rodent 2: 1-11 IFU/reaction ⁵
Intra-specificity against other viruses	100 % ⁶
Inter-specificity (against mouse, rat, hamster, human genomic DNA)	100 % ⁶
Repeatability (intra-assay-precision)	100 %
Intermediate precision (intra-laboratory-precision)	100 %

Modular Concept

Both modules, ViroInspect® Rodent 1 and ViroInspect® Rodent 2, can be used in conjunction to analyze samples for a broader panel of contaminating viruses.

⁴The Limit of Detection (LOD) of ViroInspect® Rodent 1 was determined with one DNA and one RNA model virus in presence of CHO cell culture samples processed under fermenter conditions (10 mL samples with a total of 1,0E+08 cells/reaction).

⁵The Limit of Detection (LOD) of ViroInspect® Rodent 2 was determined with quantified reference virus stocks and in one case with reference plasmids containing the specific target sequence in presence of CHO cell culture samples.

⁶Experimentally verified specifications of bioinformatics studies. Intra-specificity was bioinformatically verified and tested experimentally with reference strains/plasmids of the target viruses on the respective chip modules.

Ordering Information

REF	Description	Tests per Kit
467 011	ViroInspect® Rodent 1 Identification of Rodent Parvoviruses, PCV 1/2, Vesivirus 2117 6 tests/slide	48
467 015	ViroInspect® Rodent 2 Identification of Rodent Norovirus, Beta Coronavirus, Pneumovirus, Rubulavirus, Respirovirus, Cardiovirus, Rotavirus, Orthoreovirus 6 tests/slide	48



Figure 3: ViroInspect® Kit content



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