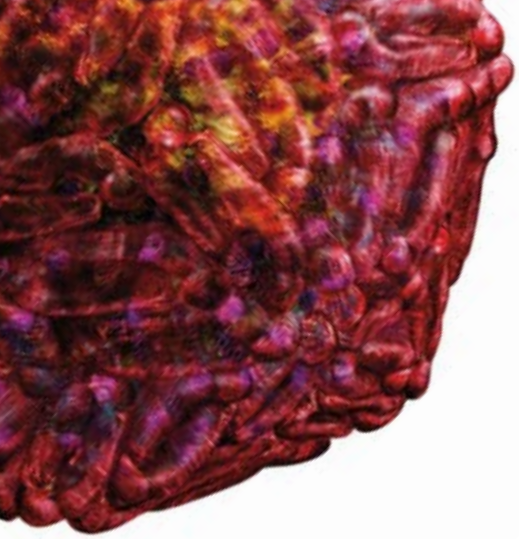

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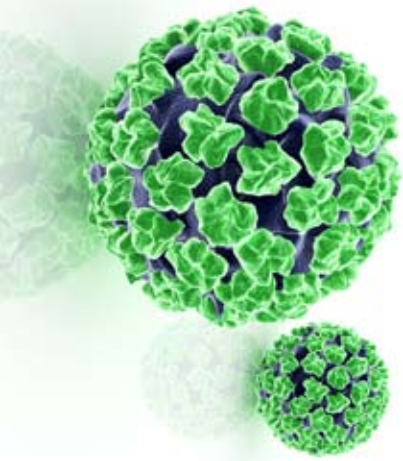

Portfolio of Services

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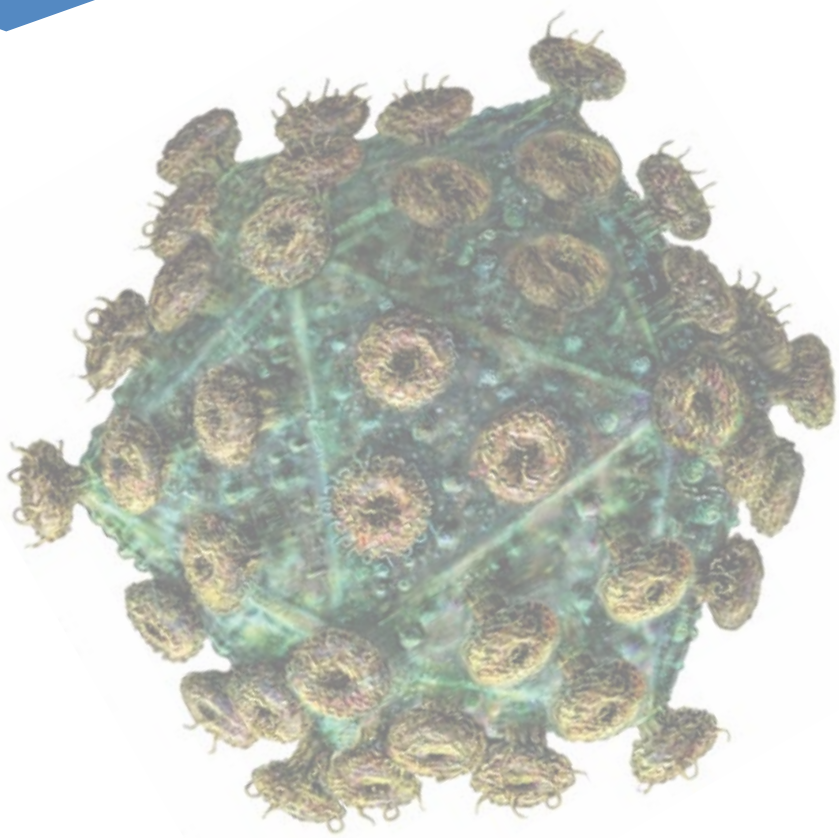

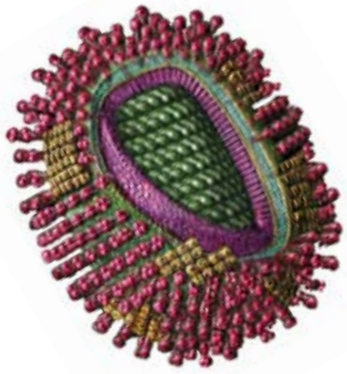

Table of Contents

Cell Banking and Cell/Virus Bank Characterisation

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The creation of a regulatory-compliant cell and virus banks is an essential component in the production of well characterised biopharmaceutical products. The use of qualified cell/virus banks provides opportunity to detect and identify (and thereby exclude) possible contaminating adventitious agents such as viruses, bacteria and mycoplasma prior to their use in manufacture. By establishing a cell/virus bank, the manufacturer can ensure that a uniform population of cells/virus is preserved, and then used as start material for each manufacturing campaign. Also of importance is ensuring that the integrity of the cell/virus bank is maintained and that a sufficient supply of material is readily available for the life span of a product.

ViruSure is dedicated to meeting your manufacturing needs for the production of cell banks for recombinant biopharmaceutical vaccine or gene therapy vector production. Our significant technical and regulatory expertise provides you with the project management and support you need for efficient product development and with guidance you can trust to ensure regulatory approval.

Our cell bank and virus bank manufacturing services include:

- Research bank pre-testing
- Manufacture of the Master and Working Cell Banks or Virus banks to GMP standards (in collaboration with qualified partners)
- Characterisation of the cell or virus banks

The process of banking is normally divided into various distinct phases resulting in the production of well characterised banks suitable for biopharmaceutical production

Research Bank Pre-evaluation

Prior starting with preparation of the Master Cell Bank (MCB) or Master Virus Bank (MVB), it is important to ensure that the research bank, which will be used as seed for the MCB/MVB, meets minimum quality standards with respect to sterility and contamination with mycoplasma. It is also advisable at this stage to include an evaluation of the cell viability, growth and protein expression levels to ensure that no significant problems are encountered when preparing the MCB.

Master Cell/Virus Bank Production

Production of the Master Cell Bank (MCB) or Master Virus Seed (MVS) must be performed to GMP standards and is considered in many guidelines as the point in the manufacture of a biopharmaceutical product where the requirement for GMP really starts. ViruSure's audited subcontractors for cell banking are fully compliant with current GMP requirements and are audited on a regular basis by our Quality Assurance group to ensure that high standards are maintained. Following establishment of the MCB/MVS, the bank is subject to a battery of characterisation tests to confirm identity and purity of the cell or virus bank.

Working Cell Bank Production

Early stages of clinical development are often performed using the MCB or MVS as the demands on the cell or virus bank are not so high. However, for later stage development, it is usual to establish a Working Cell/Virus Bank (WCB/WVS). As most of the characterisation is performed on the MCB/MVS, testing and release of the WCB/WVS is normally less extensive.

Characterisation of Cell or Virus Banks

Characterisation of the MCB/MVS and WCB/WVS must also be performed to GMP/GLP standards. The battery of tests to apply is often adapted depending on the type of cell line or virus seed under development, and ViruSure is happy to provide advice on the testing likely to be

requested by regulatory authorities. In addition to characterisation of the MCB/MVS and WCB/WVS, ViruSure can also offer testing of the End of Production Cell Bank (EPCB).

Typical tests performed for cell or virus bank characterisation include:

- Identity tests (e.g. DNA fingerprinting, Karyology)
- Sterility
- Mycoplasma/Mycobacteria
- General *In vitro* adventitious agent assay on MRC-5, Vero and a third cell line of the same species as the cell bank (for vaccine products the Phar. Eur. also requests co-cultivation with simian and human cell lines)
- *In vivo* adventitious agent testing in suckling and adult mice, embryonated eggs
- Guinea pigs *in vivo* test (optional)
- Bovine virus tests (only if exposed to bovine derived components and not previously tested)
- Porcine virus tests (only if exposed to porcine derived components and not previously tested)
- Transmission electron microscopy
- Tests for retroviruses (e.g. PG4 S+L- assay for infectious retrovirus)
- FPERT for retrovirus (not required if infectivity assay is positive)
- Specific tests for human viruses
- Specific tests for replication competent virus (e.g. for gene therapy vectors)
- Other virus specific tests dependent on the source of the cell or virus or exposure risks either prior to or during banking
- Genetic stability testing

Interference Tests

The test samples (cell and virus banks) have the potential to interfere with the test system. Especially for virus banks, which may infect the detector cell line and cause destruction of the cells. Even for cell banks, the proteins expressed by the cell bank may cause interference. It is therefore important to always control for any interfering effect from the sample matrix, and as it is impossible to interpret an assay without appropriate interference controls, ViruSure includes as standard interference controls with all of its assays. If interference is observed, we are able to assist in developing strategies to overcome such interference, either by dilution of the test sample, or through more complex procedures (e.g. ultra-centrifugation or neutralising antiserum for virus containing sample).

ViruSure has significant experience in the handling and testing of even extremely difficult to test samples (e.g. interferon or cytokines expressing cell lines) and can quickly identify the most appropriate processing of the test sample to overcome any interfering effects. We are also able to assist in the preparation of neutralising antiserum (which needs careful production to miminise risk of antibodies against potential adventitious viruses).

Advanced Therapy Medicinal Products (ATMPs)

ATMPs are an emerging field of medical products for human use, very often in regenerative medicine, but also in combined cell/drug therapies. These include gene therapy products, somatic cell therapy products and tissue engineering medicinal product. Their complexity and historical occurrence of adverse events has precipitated the requirement to develop ever more elegant test systems in order to assure product safety. Services offered in the Scope of Pathogen Safety Testing of ATMPs:

- Virus & Prion Safety Risk Assessments
- Banking services
- QC/Safety testing using established assays.
- Development of customised QC/Safety testing solutions

GMP Cell/Virus Bank Storage

ViruSure is also able to assist customers in organising the GMP storage of cell banks (in either dedicated or shared tanks) which includes temperature monitoring and yearly reports for quality purposes. For more details about the cell bank characterisation or GMP storage services please see pages 9 to 15 of this brochure.

The design of virus clearance studies is the subject of numerous regulatory guidelines, and so it is no surprise that the design and implementation of virus removal receives significant scrutiny during the review process for biopharmaceutical products. ViruSure is recognised as a world leader in designing regulatory compliant virus clearance studies and we work closely with our customers to ensure that all significant compliance aspects are covered.

Including Appropriate Controls

Virus assays are sensitive biological systems, and are easily influenced by the matrix in which the virus is titrated. It is important therefore, to control for any potential impact of the matrix on virus titre. For samples spiked with a high titre of virus, the sample itself will be diluted significantly before the end-point virus titre is reached, and at such dilutions any potential impact of the matrix on virus titre is likely to be negligible. Where present, interference will be observed for samples containing low concentrations of virus, and thus interference testing at ViruSure is always designed to investigate possible effects at or around the end-point titre of the virus. A failure to appropriately investigate interference at or around the end-point titre may result in over-inflated virus reduction factors.

Some virus spikes need to be prepared in media containing foetal bovine serum. It is advisable therefore to perform control mock-spiked experiments (i.e. using a comparable matrix to that found in the virus spike) to assess any potential impact of the spike material on the performance of the process down-scale. One recommendation is to combine such experiments with validation experiments demonstrating the validity of the downscale, which will in any event be required in support of the virus clearance study.

Validation Requirements for Investigational Products

One of the most significant clarifications in the latest CPMP guidelines on virus safety for Investigational New Products (IMPs) is to define where and how manufacturers can apply a reduced package of virus clearance studies. The question of the extent of validation for virus removal required for IMPs has never been clearly addressed in regulatory guidance like e.g. the ICH Q5A document. Validation requirements for products going into early phase clinical trials has usually followed the approach of validation with a Retrovirus model often together with a more robust model virus such as a Parvovirus (e.g. MMV or PPV) and this is now the accepted approach for products even as far as Phase III clinical studies. Parvoviruses are often referred to as a worst case challenge for virus removal (they are one of the smallest virus families and demonstrate a high resistance to inactivation procedures), and experience has shown that processes demonstrating robust removal of MMV or PPV are usually effective for the removal of other virus models or families. Thus the inclusion of e.g. a virus filtration step, effective for Parvovirus removal, will also demonstrate effective removal of all other viruses to which it is challenged. It should be noted that manufacturing processes which fail to include orthogonal dedicated effective virus removal steps will likely be subject to more intense regulatory scrutiny, and may therefore precipitate the requirement for additional virus clearance studies with other model viruses in order to provide sufficient assurances of safety. For product licensure, then validation of virus clearance according to ICH Q5A requirements is always required.

qPCR and Virus Clearance

Recent years have seen significant increase in the use of molecular based method (i.e. qPCR) in place of infectivity based assays for virus clearance studies. qPCR data can often enhance the interpretation or understanding of the mechanism of virus clearance

(i.e. either partitioning of inactivation). qPCR is often used in the context of validation of chromatography steps where the virus is inactivated as a result of the elution step (e.g. for Retroviruses and Protein A affinity chromatography together with low pH) and in nanofiltration where the virus under study may be neutralised by antibodies present in the product matrix or potentially inactivated by detergents (if present).

A number of aspects need careful control when using PCR in the context of virus removal/inactivation studies, including:

Interference controls: Controls must be included both for recovery over the extraction as well as for the PCR reaction itself to demonstrate that the sample is not interfering with detection of the viral nucleic acid

Particle to infectivity ratio: In a viral clearance study, it is useful to know if the measured removal is of non-infectious particles or infectious particles. The system should therefore be validated against an infectivity assay wherever possible.

Is the mechanism inactivation or removal? Most inactivation procedures will not result in the degradation of the nucleic acid. However, for some viruses, inactivation may expose the nucleic acid to an environment where it is not stable, and so controls to evaluate this should be implemented.

Providing these aspects are carefully controlled, qPCR assays for virus quantification can provide useful supplementary data for virus clearance studies. ViruSure has significant experience in the application of qPCR in virus clearance studies, along with the regulatory expectations where it is used in a virus clearance study.

Which Step to Validate?

ViruSure has experience in validating a wide range of potential virus clearance steps, including filters from all of the major nanofilter manufacturers, chromatography steps, inactivation steps (e.g. solvent/detergent, alcohol, heat, low or high pH ....), gamma or other irradiation technologies (e.g. UV), precipitation and many other steps. Furthermore, ViruSure has extensive experience with the inactivation of viruses in solid start material (e.g. tissues) as well as solid surfaces (stainless steel coupons). We are also able to provide valuable support for customers on the effectiveness of different procedures using our extensive in-house database of studies.

If you would like to know more about our virus clearance testing services, please contact us at virus_safety@virusure.com.

A Selection of Model Viruses Available for Clearance Studies at ViruSure

The selection of viruses for virus clearance studies is dependent on the risk profile of the product. The selection should cover a range of physico-chemical properties, including those viruses with the high resistance to inactivation, and include those viruses which are potential contaminants. The viruses marked with an * are almost universally selected for the validation of virus clearance in recombinant biopharmaceutical products. Other virus types may also be considered if the risk profile of the product suggests potential contamination with members from that particular virus family. All of the above listed viruses are available for use in spiking studies at ViruSure. This table however is not intended to be an all inclusive list of model viruses. Please enquire if there is a specific virus model you require for your studies.

Target Virus Class	Example Model Viruses	Genome	Size (nm)	Envelope?	Resistance
Retroviruses	Murine leukaemia virus (MuLV) *	2x ssRNA	80-110	Yes	Low
Herpesviruses	Pseudorabies virus (PRV) * Bovine Herpesvirus (BHV)	dsDNA	120-200	Yes	Low-Med
Reoviruses	Reovirus type 3 (Reo3) *	dsRNA	60-80	No	Med-High
Parvoviruses	Mice minute virus (MMV) * Porcine Parvovirus (PPV) * Parvovirus B19 (pB19)	ssDNA	18-22	No	High
Picornaviruses	Hepatitis A (HAV) Encephalomyocarditis virus (EMCV)	ssRNA	28-30	No	Med-High
Calicivirus	Vesivirus 2117	ssRNA	35-40	No	Med-High
Influenzavirus	H1N1, H5N3	ssRNA	80-120	Yes	Low
Bunyavirus	Cache valley virus (CVV)	ssRNA	100	Yes	Low

Prion Clearance Services

With the revised EMA position statement on Creutzfeldt-Jakob disease (CJD) and the safety of plasma and urine derived medicinal products (Position Statement on Creutzfeldt-Jakob Disease and the Safety of Plasma- and Urine-Derived Medicinal Products. Committee for Proprietary Medicinal Products, 2004, EMEA/CPMP/BWP/2379/02 Rev. 1), there is an increased pressure on plasma product manufacturers to perform prion clearance studies.

Current trending of new vCJD cases has shown a declining but continuing incidence, not just in the UK but also in other European countries. However, the emergence of other TSE agents like atypical BSE or Chronic Wasting Disease (CWD) continues to challenge the systems for ensuring the safety of biopharmaceutical products with respect to TSE agents. Even with a declining number of vCJD cases, it is clear that a risk of potentially infected blood donors contributing to the plasma pool will continue for some time. Furthermore, the EMA guidance document on the investigation of manufacturing processes for plasma-derived medicinal products with respect to vCJD risk (Guidelines on the Investigation of Manufacturing Processes for Human Plasma-Derived Medicinal Products with Respect to vCJD Risk, Committee for Proprietary Medicinal Products, 2004, CPMP/BWP/CPMP/5136/03) provides a framework assisting the design of prion clearance studies, and applies many of the principles that have served virus validation studies well over the years. Investigations for prion removal or inactivation however still present challenges not encountered with virus studies. It is clear that for prions, we do not yet have the level of understanding of the nature of infectivity in blood that would allow such certainty of study design and interpretation. There is still significant debate over the form of spike to be used for prion clearance studies, particularly for human blood or plasma-derived products where the nature of the infectious unit present in blood is still to be fully characterised.

In instances of vCJD implicated products, it is clear that any product manufactured from such plasma pools would be recalled in compliance with regulatory guidance. Recall events for vCJD, however, can also have significant consequences for subsequent batches of product manufactured using the same equipment. Such events highlight the importance of prion inactivation or clearance studies, and particularly the importance of demonstrating:

1. Effective prion removal by the manufacturing process, such that in the event of prion carry-over, any risk to subsequently manufactured product can be considered small
2. Effective cleaning and sanitisation procedures to minimise the potential of vCJD carry-over from previous batches

ViruSure's experience with assessing and ensuring the safety of biological products with respect to TSE agents stretches back more than 20 years to when the first cases of vCJD were identified. With our extensive experience we are able to assist our customers with the preparation of risk assessments in relation to TSE agents as well as in performing prion clearance studies. Our experience includes validation of all of the major classical prion clearance steps, including small nanofiltration, precipitation and chromatography as well as sanitisation steps (including coupon sanitisation studies- see next page). We are highly experienced in overcoming interference often seen with difficult samples like immunoglobulins when testing for prion removal using Western blotting. By overcoming this interference (through carefully controlled concentration methods) we are able to maintaining assay sensitivity and ensure the best possible outcome for a prion clearance study.

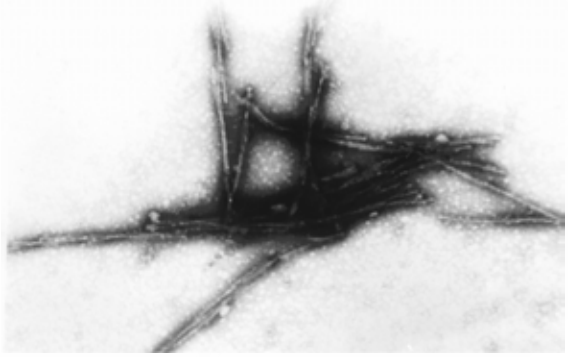
If you would like to know more about our prion clearance testing services, please contact us at virus_safety@virusure.com.

Prion Sanitisation

Published studies with prions have demonstrated that quality and nature of the spike preparation and microenvironment of the agent can have a dramatic effect on the capacity of an inactivation procedure to inactivate prions. Procedures which result in "fixing" of the prion agent, such as drying, treatment with organic solvents or cross-linking with aldehyde based disinfectants results in an increase in the proportion of prion protein demonstrating resistance to inactivation.

The observation of increased resistance to inactivation following certain procedures has implications for the disinfection of biopharmaceutical equipment or surgical instruments suspected of CJD agent contamination. Current practice for the GMP cleaning of biopharmaceutical equipment require that potential for carry over of product and contaminants must be minimised, that cleaning procedures are validated, and where components with an identified risk from TSE agents are used, then prion cleaning/inactivation data may be required. Sanitisation therefore becomes a significant topic for discussion for multi-product facilities, for slaughterhouses collecting raw components for subsequent manufacture and where re-dedication of equipment previously exposed to potential risk material is performed.

ViruSure has extensive experience in performing cleaning validation studies using stainless steel coupons or other surface materials.



Scrapie Associated Fibrils, a characteristic of prion contamination in high titre brain derived preparations (picture kindly provided by Dr Robert Somerville, Institute for Animal Health, Edinburgh, UK).

ViruSure's Prion Clearance Services

We are able to offer a number of services to assist you in meeting your prion removal requirements:

Western blot testing services

With our fully validated semi- quantitative Western blot assay for 263K hamster adapted Scrapie (a model spike agent recommended in guidelines), we can perform investigational studies on the ability of manufacturing processes to remove or inactivate prions in compliance with EMEA, FDA and JMHLW requirements.

In vivo prion bioassays

We operate a fully validated animal facility equipped for working with rodent adapted prion strains. We can therefore also perform *in situ* cleaning studies, using stainless steel wires exposed to high titre prions (based on the procedure as described by Flechsig *et al.* (Transmission of scrapie by steel-surface-bound prions. Mol Med. 2001 Oct; 7(10):679-84)

TSE model agents available at ViruSure

We are able to work with many of the available rodent adapted prion strains, but currently most studies at ViruSure are designed and performed using the 263K hamster adapted strain of scrapie prions. This agent has been widely used in prion inactivation/removal studies, and is widely accepted as a suitable model for other TSE agents in such studies.

In vitro Testing Services

ViruSure's Tech Gate facility has been established with the goal of providing high quality *in vitro* testing for biopharmaceutical products. A strict segregation concept together with full ICH compliant assay validation ensures trustworthy test results. All tests are performed as standard (i.e. no additional charge) with appropriate matrix interference controls where necessary. A range of *in vitro* tests are offered by ViruSure as summarised below

Characterisation of Cell-Banks & Virus-Seeds

Cell banks or virus seeds used to produce biologics and the biotechnology products derived from them can all be potentially contaminated with adventitious viruses. Cell culture tests are used in detecting a wide spectrum of viruses that are either cytopathic, haemadsorbing or haemagglutinating , or by probing with specific fluorescent antibodies.

Adventitious agent testing plays an important role in the lot release of recombinant biopharmaceutical, vaccine and ATMP products and is required at the following stages of biopharmaceutical product development and production:

- MCB, WCB and EPCB
- Master virus seed banks
- Production lot release

In vitro Adventitious Agent Test

In Adventitious Agent Testing (AAT), samples are inoculated onto 3 types of monolayer cell culture:

1. Human diploid cell line (e.g. MRC-5)
2. Simian kidney cell line (e.g. Vero)
3. Same species/tissue type as that under test

The test can be performed as a 14 or 28 day assay. If no CPE is observed cells and supernatants are tested for haemagglutination and haemadsorption at the end of the assay. Furthermore, where a client specific cell line is required as a detector system , this can impact significantly on customer timelines and costs. ViruSure has extensive experience in the development and validation of in vitro cell culture assays, allowing our customers to minimise any potential impact on their projects.

Testing of Animal-Derived Products and Recombinant Products for Virus Contamination

The term in vitro testing is applicable both to tests performed on the production material as well as to the testing of raw materials. For example, guidelines have been established in both Europe and the US FDA for the testing of bovine serum prior to use in manufacture. This includes tests on permissive bovine cell lines with specific end point detection for Bovine Viral Diarrhoea virus, Bovine adenovirus, Bovine parvovirus, Bovine respiratory syncitial virus, Reovirus, Bovine paramyxovirus, Rabies and Bluetongue virus. Similar tests are available for Porcine derived contaminants.

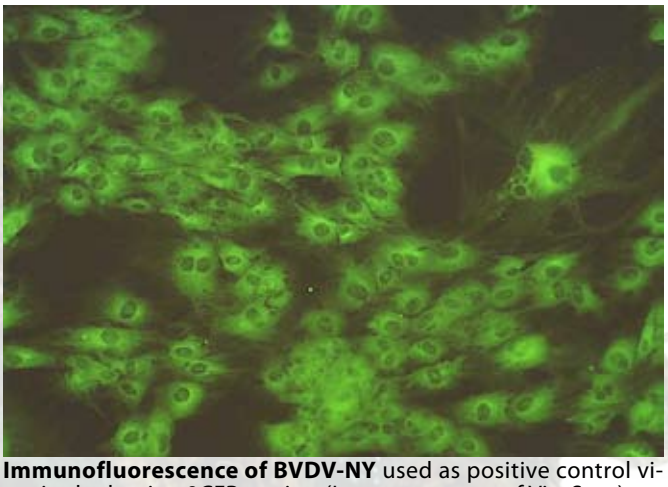
In vitro 9CFR Testing

The *in vitro* 9CFR Assay is used to detect potential contamination from exposure to animal derived components. Samples are inoculated onto 2 types of monolayer cell culture:

1. Simian kidney cell line (e.g. Vero)
2. Bovine/Porcine Cell line (e.g. BT/ST)

At the end of the 21 day observation period cells are evaluated for CPE and haemadsorption and probed with specific immunofluorescent antibodies. As an alternative to infectivity testing, bovine or porcine viruses can also be detected using our

standard package of qPCR tests (see Pages 11/12) which is able to detect a broader range of adventitious viruses.



Immunofluorescence of BVDV-NY used as positive control virus in the bovine 9CFR testing (Image property of ViruSure)

Retrovirus Testing

Retroviruses are a known and accepted viral contaminant of e.g. recombinant cell lines. Despite their acceptance, guidelines still require the characterisation of any retrovirus contamination in terms of particle type, quantification or the retrovirus-like particle load, levels of retrovirus associated reverse-transcriptase (i.e. FPERT), and characterisation of the presence of any infectious retroviruses. ViruSure offers a range of retrovirus testing services to meet the above regulatory requirements, including, retrovirus infectivity tests (S+L- assay, *Mus dunni* amplification assay ....), FPERT testing, electron microscopy for characterisation or quantification of retrovirus particles and co-cultivation assays.

Including Appropriate Controls

Cell culture assays are sensitive biological systems, and are easily influenced by the matrix in which the test is performed. It is important therefore, to control for any potential impact of the matrix on the assay system. Interference controls (test matrix spiked with positive control virus) are included as standard for all the tests described above. Additionally where procedures are required to overcome high levels of interference (e.g. neutralization using antibodies) appropriate controls are included to assess the effect of the treatment on the assay system.

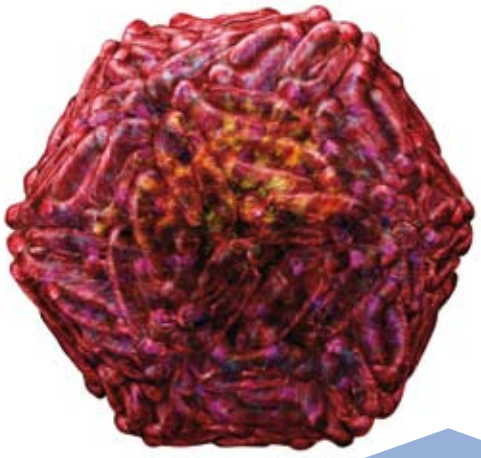
Testing of ATMPs

Advanced therapy medicinal products (ATMPs) are a rapidly developing class of human medicinal products often in the field of regenerative medicine. They can be stratified into one of the following three categories of product: gene therapy, somatic cell therapy or and tissue engineering and regenerative medicine.

The testing of ATMPs presents significant challenges not found with classical recombinant biopharmaceuticals (e.g. lack of validated virus removal steps, rapid turn-around time to product release, testing for replication competent virus vector). ViruSure has significant experience in designing and optimising the testing package for ATMP products- please contact us to discuss our full range of services and support.

Testing of Live Virus Vectors

For live virus vaccines or replication competent gene therapy vectors, issues may be encountered with cell based detection systems where the virus is able to replicate on the detector cells (either resulting in cytopathic effect, or maybe interfering with the detection of adventitious viruses). In such instances, neutralisation of the replication competent virus may be required. ViruSure offers the preparation of viral antigens for inoculation into an appropriate animal species (e.g. rabbits) in order generate neutralising antibodies, as well as the characterization of the resulting antiserum by Western Blot and the evaluation of neutralizing capacity using an in vitro or in vivo system. We have extensive experience in testing of such samples, and have developed optimised neutralisation protocols that ensure high levels of vector neutralisation.



qPCR & Sequencing Services

A range of molecular biology services are available at ViruSure to meet your biosafety testing needs, including:

- qPCR testing for specific adventitious agents
- qPCR for virus clearance studies (see Page 5/6)
- qPCR assay establishment and design
- Genetic stability (gene copy number, DNA or RNA sequencing)
- Biodistribution studies (e.g. for gene therapy vectors)
- GLP Sanger or deep DNA sequencing

Our PCR and sequencing labs have been designed to ensure a strict segregation that minimises any risk for cross contamination. For our GMP adventitious agent testing, ViruSure only works with qPCR technologies (i.e. no PCR product is analysed in gels) which eliminates the potential for contamination from previous qPCR testing.

qPCR Tests for Virus Contaminants

For certain viruses, detection in cell based infectivity systems has remained challenging, and for these viruses qPCR often offers the only viable test system for detection. ViruSure is able to offer a range of qPCR tests for common virus contaminants including Mice minute virus (MMV), Porcine circovirus (PCV1 and PCV2), Vesivirus 2117 and many others (e.g. bovine, porcine, equine, simian, insect derived ..... contaminants). Please contact us for specific virus PCR tests.

FPERT Testing for Retroviruses

As detailed in regulatory guidance, testing for Retrovirus associated reverse transcriptase (RT) should be performed using a highly sensitive PCR based approach. ViruSure has developed a highly sensitive Fluorescence based Product Enhanced RT (FPERT) test that is used for the characterisation of RT activity in vaccine and recombinant-derived biopharmaceutical products. The FPERT assay has the advantage of being more sensitive and reproducible in comparison to other RT detection methods.

qPCR Development & Assay Validation

Before developing a new qPCR test, an extensive literature search, combined with creating alignments, is performed. This ensures that highly conserved regions are used for primer design, allowing the coverage of as many possible subtypes using a single PCR reaction. For less conserved viruses, it may be necessary to establish multiple qPCR assays to ensure detection of all virus types.

All qPCR assays are validated to the strictest ICH Q2 R1 requirements, and evaluate linearity, range and reproducibility, intermediate precision, specificity, LOD/LOQ, accuracy and robustness. All validated assays are evaluated on a bi-yearly basis which includes an evaluation of all deviations, trending of the standard curve which is performed for each PCR test, as well as a search for new sequences that have appeared since the original assay development that might require an assay redesign.

qPCR Assay Controls

Specific controls to ensure system suitability and to exclude assay interference from the test sample matrix are included as standard in all ViruSure test systems (i.e. no extra charge) according to the acceptance criteria defined by ViruSure Quality systems for PCR assays.

GLP Sequencing

Sanger sequencing by primer walking allows a precise sequence identification of long fragments with high accuracy. ViruSure's GLP compliant sequencing services include the following:

- 4-fold coverage of the target gene
- Validated systems per ICH guidelines
- Expert advice on the most appropriate sequencing strategy for your product

In addition to Sanger sequencing, ViruSure can support your next generation sequencing requirement contact us for more information.

Ph. Eur. Compliant qPCR Mycoplasma Testing

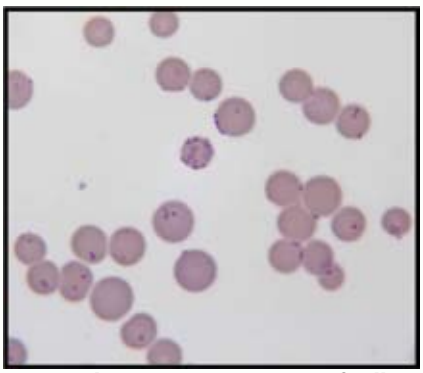
Mycoplasmas are among the biggest contaminant concerns in biological products and although Mycoplasma do not kill contaminated cells, they can have a variety of effects on cultured cells, such as altered metabolism, slowed proliferation and chromosomal aberrations, which can compromise the quality of biopharmaceutical products.

Due to their diversity, their small size, and the absence of a bacterial wall, they cannot be observed by light microscopy and are therefore difficult to detect. ViruSure has developed and validated its own Real- Time PCR detection method (nucleic acid amplification technique, NAT) following European Pharmacopeia and ICH guidelines and is using it already for routine release testing.

The Mycoplasma Assay

The assay is performed in accordance to European Pharmacopeia (EP) section 2.6.7, where NAT is now considered as an alternative to the cell culture method after suitable validation. The method detects all Mycoplasma species described in the EP, and in addition ViruSure has confirmed detection of the additional strains requested by the Japanese Pharmacopoeia (JP):

- Mycoplasma gallisepticum (EP)
- Acholeplasma laidlawii (EP + JP)
- Mycoplasma fermentans (EP + JP)
- Mycoplasma hyorhinis (EP+ JP)
- Mycoplasma orale (EP + JP)
- Mycoplasma synoviae (EP + JP)
- Spiroplasma citri (EP + JP)
- Mycoplasma pneumoniae (JP)
- Mycoplasma arginini (EP + JP)
- Mycoplasma salivarium (JP)



Mycoplasma contamination of cells revealed by staining

The NAT uses a TaqMan Assay where the primers and the probe bind to a highly conserved region of the mycoplasma genomes, the 16S rRNA. Detection of all mycoplasma strains has been validated and met the European and Japanese Pharmacopeia defined Limit of Detection for the Mycoplasma qPCR assay of at least 10 CFU per ml.

Mycoplasma qPCR Advantages

The qPCR method is a highly sensitive method and compared to the standard culture method, which takes 28 days, it is an extremely rapid method where even testing of multiple samples can be done within a few days.

To find out more about these services, as well as our full range of biosafety testing service, please contact us via email at: virus_safety@virusure.com.

ViruSure's newly established *in vivo* facility is fully GMP and GLP compliant and offers a modern, state of the art laboratory animal housing concept for performing in vivo testing. The laboratory was specifically designed to meet ViruSure's needs and comprises individually HEPA-filtered ventilated cage systems for animal containment, a pass-through autoclave for decontamination procedures and laminar flow class II cabinets for safe sample and animal handling. The fully validated systems combine a strict segregation concept with optimized hygiene measures for the generation of trustable, high quality test results.

Prion Bioassays

As detailed in the latest EU guidance on the performance of prion clearance studies, the titration of samples from prion clearance studies in hamsters and/or mice may be required where insufficient assurances around the level of prion removal can be provided by *in vitro* systems for prion detection, such as Western blot analysis. *In vivo* titration is still to date the most sensitive method for determining prion reduction factors of manufacturing processes. Due to the slow development of TSE disease, these in vivo assays can require observation periods of 6 months to 1 year.

In vivo adventitious agent testing

In vivo assays for the detection of virus contaminants are comparable to the general *in vitro* adventitious agent assays. The assays are performed in animal models by the inoculation of the Test Sample into adult mice, suckling mice, embryonated chicken eggs and/or guinea pigs. The end point of the test is the detection of pathogenic viruses either by observation of clinical symptoms or via heamagglutination. This test is generally only required for the characterisation of cell banks, and is not routinely performed on production harvests (although this test may be requested for certain classes of product)

Tumorigenicity Testing

Tumorigenicity is defined as the potential of cells to form tumors when inoculated into animals (generally an immunosuppressed animal model). Tumorigenicity is a characteristic of immortalized cells themselves, rather than of any agents or components present in them. Tumorigenicity testing is required by FDA, WHO and EU guidelines for vaccine cell substrates used for the manufacture of human vaccine products. Generally, 10^7 test cells and positive control cells (with known tumorigenic potential) suspended in 0.2 mL are administered by the subcutaneous route into immunosupressed mice. Usually, cells from the end-of-production passage level are used for this testing. The number of animals developing tumours are compared with those that have received a positive control tumor cell line (e.g. HeLa). Cell lines demonstrating tumorigenic potential may require additional characterization via oncogenicity testing or characterization of the size of residual DNA fragments.

Oncogenicity Testing

Oncogenicity testing is designed to assure that agents, with the potential to immortalize cells and endow these with the capacity to induce tumors are not present in a cell substrate. If a vaccine is manufactured in a cell line, that has a tumorigenic phenotype, this cell line may have a higher theoretical risk of containing oncogenic substances (e.g. oncogenes or oncogenic viruses). All tumours that develop in an oncogenicity test are examined for the presence of DNA from the species of which the cell line was derived from, as well as from the animal in which the oncogenicity testing was performed.

Antibody Production Assays (MAP/ HAP/RAP)

These in vivo assays are performed in mice, hamsters or rats to detect potential contamination of test materials with specific adventitious agents of rodents. The Test Sample is inoculated via several different routes into the animals and after a defined observation period blood is collected and analyzed for the presence of antibody against a defined list of antigens relevant for the specific species.

Bio-Distribution Studies

With the increasing use of gene therapy technologies (i.e. ATMPs) as detailed characterisation of the vector in an *in vivo* system is often required. In additional to any vector design aspects, the administration route and formulation can greatly influence toxicity of a gene therapy vector and should be evaluated in bio-distribution studies. The aim of such studies, is to identify target organs for toxicity and reversibility. The goal therefore of bio-distribution studies is to identify the target organs for toxicity and/or vector spread, including germline transmission. Most biodistribution studies utilise qPCR for quantification of the vector in different tissues, and sensitivity and specificity of the qPCR test become an important component of the study design. The use of spike or internal controls is recommended to control for PCR inhibition or interference with extraction.

Other In vivo Studies

Our facilities and staff are able to assist with the design of a range of in vivo study designs to suit your requirements. If you have a specific design not detailed above, please contact us to discuss if we are able to assist in meeting your needs on virus_safety@virusure.com.

GMP Storage of Cell and Virus Banks

One of the important aspects in the production of a biopharmaceutical product is the establishment of master and working cell-, bacterial- or virus banks to ensure batch to batch reproducibility. Following an appropriate characterisation and release, the storage of these cell- bacterial banks (MCB/WCB) or virus- banks (MVSS/WVSS) up to their point of use is a fundamental and essential aspect of the GMP Banking System. A Qualified GMP compliant storage environment protects the Cells, Virus or bacterial Stocks and guarantees constant storing conditions thus ensuring integrity of the biological material over the life span of the product. While a part of the stocks can be stored at the production facility, the EU GMP guideline advise to split the banks in different locations to avoid the risk of total loss. GMP storage encompasses the following attributes:

- Fully qualified storage units that use a segregation policy/strategy for different biological materials
- Controlled access to storage zones restricted to authorized personnel.
- Every storage container is adequately sealed, clearly labelled and kept at an appropriate temperature.
- Storage temperature is continuously recorded and connected to a validated alarm system that triggers a 24 hr alarm call-out system whenever temperature excursions and deviations occur
- Any deviation from the set temperature limits is carefully evaluated and any corrective and preventive action taken is recorded.
- Back-up systems in the event of power failures or failures in the cooling device

Storage at <-60°C

Storage of virus and bacterial seed banks is performed according to GMP standards using the conditions detailed above. The banks are stored in fully validated and continuously temperature monitored -80 °C freezers (with an alarm setting at <-60°C).

Cell Bank Storage in Ultra-Low Freezers (<-130°C)

For the storage of mammalian cell banks that require ultra-low temperatures, ViruSure offers storage in validated and continuously temperature monitored ultra-low deep freezers. Storage in liquid nitrogen tanks bears the risk of cross contamination, especially if the vials are stored close to or in the liquid phase and not in the vapour phase. Liquid nitrogen is known to contain low levels of microbiological contaminants, and during transport and storage can become further contaminated by ice, inanimate debris, and viable microorganisms. Furthermore, the filling of liquid nitrogen tanks must be carefully controlled, and failures of e.g. automated filling systems can result in either too little liquid nitrogen or an overfilling of the tank resulting in failure of the vapour phase storage conditions.

Because of these known risks with liquid nitrogen, an alternative strategy which avoids the requirement for liquid nitrogen completely is the use of ultra-low freezers, which operate efficiently down to cryogenic temperatures. Such systems are fully compliant with GMP storage requirements and result in a stable and well controlled low temperature for the long-term maintenance of GMP cell and virus banks. Ultra-low freezers are compliant with ICH Q5D, EU and WHO requirements for GMP cell bank storage. Many companies, including ViruSure, have been storing cell banks using such ultra-low freezers for a significant period of time without any impact on viability or performance of the cells. The use of ultra-low deep freezers therefore presents a safer and more secure environment for GMP cell banks with none of the handling issues associated with liquid nitrogen.

The ViruSure Quality Management System

At ViruSure our success can only be assured through a total commitment to test quality, customer service and regulatory compliance.

Quality must be planned from the beginning through completion for any successful study or analytical test. With this goal, a state-of-the-art risk based Quality System as designed and implemented, which covers not only the legal aspects of GMP, GLP, Health and Safety etc, but also components of ISO 9000 in order to ensure a strong focus on customer service.

All employees along with Management are actively involved in the continuous improvement of the quality systems.

We commit and ensure that from preparation of the contract through to completion of the audited final report or Certificate of Analysis and beyond, that all work will be performed to the highest quality. Our QA group is actively involved in all aspects of the company, and interested parties are welcome to audit our laboratory space and quality systems, which has continually received excellent feedback from our customers. ViruSure is certified by the Austrian authorities (AGES) for both GMP and GLP compliance (this includes both *in vitro* and in vivo facilities).

The established quality system includes:

- GMP/GLP compliant facilities and systems (copies of our certificates can be downloaded from our website)
- A transparent assignment of responsibilities
- Full risk assessments built into all SOPs and system manuals. These risk assessments enhance the transfer of risk management to the employees working with the systems and helps to ensure employees understand the risks associated with each system
- Documented internal and external training and authorisation of staff
- Regular internal inspections by QA experts
- Traceability on receiving, storage and analysis of test articles
- The use of only controlled and traceable materials for critical tests
- Regular audits of all subcontract laboratories or suppliers of critical reagents and materials used in tests
- Validated assays & periodic evaluation of the validation status
- Calibrated and qualified equipment and instrumentation installed through a formal IQ/OQ/PQ procedure
- System, equipment and analytical SOPs
- Controlled Study Plans and Final Reports (GLP)
- Controlled SOPs, Client Protocols and Certificate of Analysis (GMP)
- Change Control procedures
- Stringent non-conformance and OOS procedures
- Archiving of documents for a minimum of 10 years

Please contact us at **virus_safety@virusure.com** if you would like to learn more about our Quality Systems.

Quality is not a Coincidence!

Company Overview

ViruSure is still a privately owned Austrian company specialising in the pathogen safety testing of biopharmaceutical products and offers a breadth of experience as well as unparalleled customer service. Our size and independence allow us a high degree of flexibility and a rapid decision making matrix that guarantees a specific focus on our customers needs.

Company History and Overview

The company was established in 2005 with a simple goal- to provide our customers with a high quality one stop shop for all their virus and prion safety testing requirements, including GMP cell and virus banking and characterisation, virus and prion clearance studies and other in vivo tests systems such as tumorogenicity and oncogenicity testing, as well as cell based potency assays. Our studies support the licensure and development of all classes of biopharmaceutical products including recombinant biopharmaceuticals, vaccines, animal derived products, human plasma derived products and medical devices through all stages of development. Both our *in vitro* and *in vivo* testing facilities are certified to GLP/GMP standards and operated by experienced staff under a state of the art risk based quality management system.

Experience in Depth

The company was founded and is operated by a highly experienced Management Team - derived from all branches of the biopharmaceutical industry including virus safety testing companies, vaccine manufacturers, GMP manufacturing, regulatory inspectorates and experienced virologists who together bring an unrivalled experience in the virus safety testing of biopharmaceutical products. We can therefore provide an unparalleled level of expertise and knowledge to the table to assist our customers in ensuring that the testing strategy for their product fulfils all necessary scientific and regulatory requirements. Together with the client, we can advise and develop a range of product specific assays, including:

- Specific qPCR Assays
- Cell based assays
- Neutralization protocols for Virus based vaccines & gene therapy products.
- Replication Competent Lentivirus (RCL)
- Fluorescence based Assays
- Or any combination of the above

With our unrivalled experience in this highly specialised area of testing you can have a high level of confidence that the testing strategy we recommend will fulfil all significant regulatory requirements. Studies performed by ViruSure have been accepted by regulatory agencies world-wide, including EMA, FDA, PEI, TGA, JMHLW, MFDS (Korea) and other authorities. ViruSure is experienced in supporting our clients in regulatory submissions or if necessary visiting the authorities together with the client.

Unrivalled Customer Service

Our strong commitment to scientific excellence, quality and customer service has established ViruSure as a preferred supplier for biopharmaceutical companies throughout the world. At ViruSure, customers come first, and we strive to ensure that we deliver a high quality service. Our state of the art risk-based Quality Management System enables an environment where ownership for quality of the service is designed from initial contact through development of a strategy and study performance and reporting. A strong emphasis on quality is present throughout. To take advantage of our customer focussed and professional service, please contact us through our website at **www.virusure.com**, or via email: **virus_safety@virusure.com**.

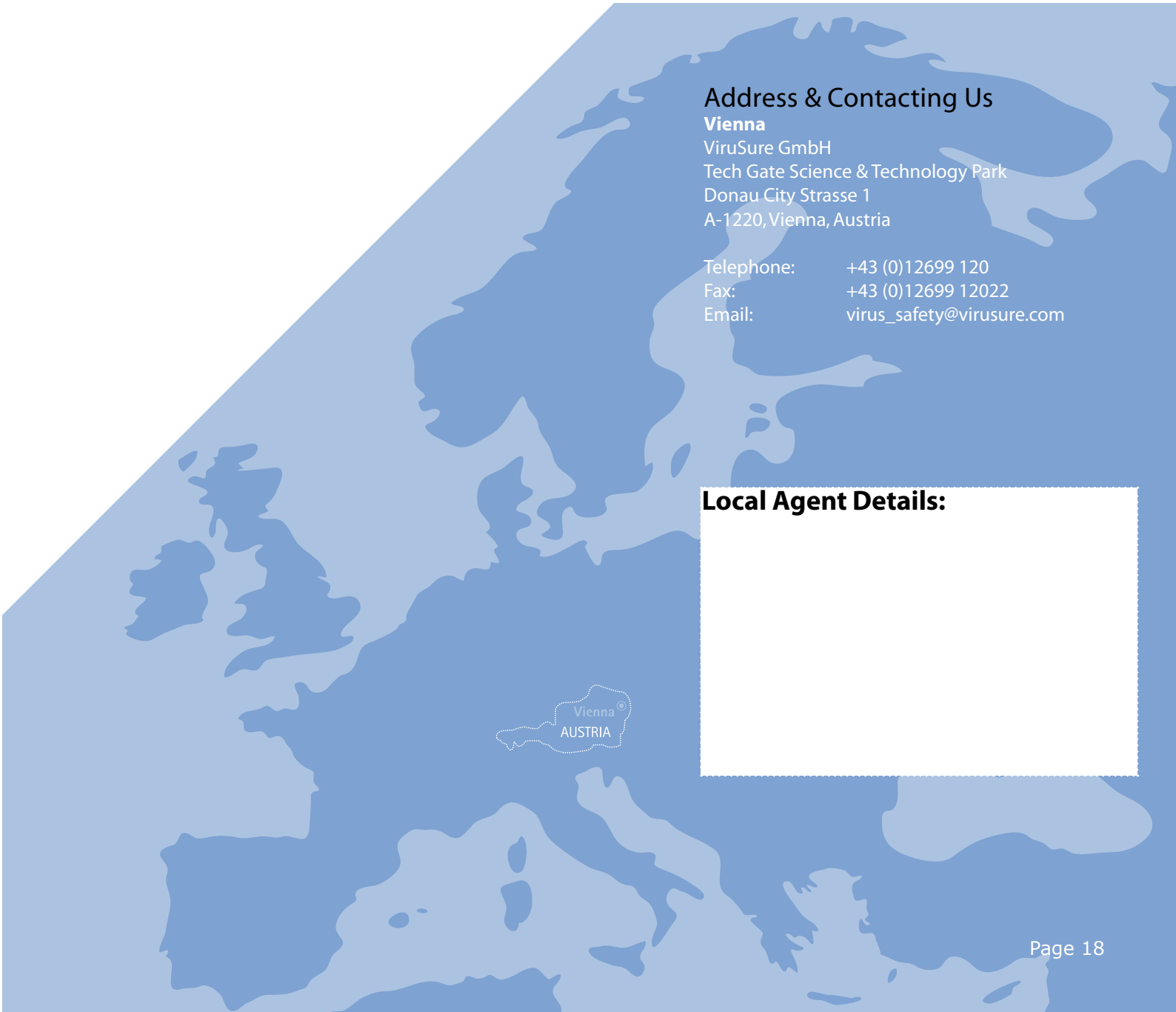
Key Staff

Andy Bailey; Ph.D.- Operations Director & founder
Walter Tabotta; Ph.D.- Head of Testing Services & QP
Angelika Spreitzhofer; M.Sc. - Quality Manager
Katrin Weixelbaumer; Ph.D.- Manager, *In Vivo* Studies
Katy Lorineau; M.Sc.- GMP Storage manager & quality specialist
Tiffany Avory- Manager; GMP Testing Services
Jürgen Ebner; Dipl. Ing.- Manager; Virus Clearance Testing Services
Natascha Hodosi; Dipl. Ing.- Manager; PCR & Sequencing Testing Services
Alexandra Weidler; Dipl. Ing. (FH)- GMP Project Coordinator & QP

VIRUSURE
VIRUS & PRION TESTING
.... just a quality better!

Business Development

Ralf Klein; Ph.D.- Business Development Manager
Luis Raiado; Ph.D.- Business Development



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