BioReliance

Biologics Safety Testing Services

Gene and Cell Therapy Solutions

Custom and Routine Assay Capabilities

BioReliance provides a comprehensive range of assays and services to support every stage of gene and cell therapy development – from viral vector manufacture to a broad spectrum of biosafety testing in accordance with designated regulatory guidelines. Whether your endpoint for gene transfer is targeted for vaccines or therapeutics, quality GLP and GMP testing is offered with leading turn-around times and scientific expertise.

Table 1: Next generation transfer vectors

Commonly used gene transfer vectors	
Viral Vectors	Non-viral based vectors/delivery
Adenovirus	Naked DNA (plasmid)
γ - Retrovirus (MuLV)	Liposomal complexes
Adeno-associated virus (AAV)	Polymer complexes
Lentivirus (LV)	PEGylation
Pox/Modified Vaccinia Ankara (MVA)	Dendrimers
Herpes Simplex Virus (HSV)	Nanoparticles
Alphavirus (e.g., Sindbis, Semliki)	Cell permeable peptides
Sendai Virus (SeV)	Microbial/bacterial

Gene and Cell Therapy based medicines are experiencing a resurgence due to the introduction of 'next generation' transfer vectors (Table 1) which have demonstrated improved safety and efficacy. BioReliance partners with our gene therapy focused clients to apply our technical testing expertise and the highest level of quality to meet our clients' testing needs.

Patient cells are often extracted, expanded, and transduced using gene therapy vectors and then the modified cells are re-implanted in the patient for therapeutic effect. In this circumstance, the modified cell is the drug product rather than the gene transfer vehicle and carries with it distinct regulatory challenges. Regulatory approval for a gene-modified cell therapeutic product arrived with EMA approval of a therapeutic use for Adeno-associated virus (AAV)-mediated delivery of a treatment for lipoprotein lipase deficiency. Stem cell therapies[1], particularly those associated with genetically corrective endpoints, are being investigated in numerous clinical trials. In summary, gene and cell based therapies go hand-in-hand.

Given the complexities associated with new technologies, especially with 'first in class' applications, efficient and effective communication channels are frequently the deciding factor in the success of a testing or manufacturing program. Our charter at BioReliance is maintaining our clients at the center of our organization. We do this through effective communication via integrated project management, account management, development services, and laboratory operations. Our people are your primary connection to our organization - all are trained and dedicated to providing you the best quality of service.

Routine and custom assay development services to support the unique testing needs of Gene and Cell Therapy Clients

GLP or GMP testing with leading turn-around times and reporting

Consultative regulatory compliance guidance offered by industry experts

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Table 2: Recombinant Engineering of Gene and Cell Therapies

Viral/nucleic acid manufacturing platforms	Vector
Bacteria (e.g., *E. coli*)	DNA plasmid, RNA transcripts, Transposons (sleeping beauty)
Avian (e.g., CEFs, EB66, embryonated eggs)	Avipoxvirus, MVA, Alphavirus (Sindbis, Semliki), Sendai
Mammalian Human (e.g., HEK293/T, HeLa, PER.C6®) Primate (e.g., Vero, Cos-7) Rodent (e.g., BHK-1, Psi-2, PA317) Other (e.g., Canine – MDCK)	Adenovirus, AAV, Lentivirus, MuLV
	Herpes Simplex Virus (HSV)
	MuLV/ γ - retrovirus
	Influenza, virus-like particles (VLP)
Insect (e.g., *S. frugiperda*, Sf9/21; *T. ni*, High Five)	Baculovirus/AAV, BV/Adenovirus, BV/Lentivirus, BV/VLP

Our state-of-the-art facilities are well equipped to meet your gene and cell therapy manufacturing and testing needs on a global scale (Table 2). Our Carlsbad (CA) and Rockville (MD) facilities represent our US Centers of Excellence in viral product manufacturing and biosafety testing, respectively – with capabilities of cGMP grade bulk virus preparation augmented by industry leading analytical testing services. Coupled with our BioReliance UK GMP Manufacturing and testing facility, we have worldwide capabilities and capacity to successfully support your projects.

Testing Capabilities

BioReliance offers a comprehensive array of assays in support of gene and cell based therapies – both routine (adventitious agent testing, mycoplasma, sterility) and custom designed (identity, potency assays) to meet all clinical and regulatory compliance standards (Table 3). These assays are supported by dedicated Lab Operations and Development Services groups to ensure the highest quality and turn-around-times.

Table 3: Gene and Cell Therapy Testing

Test	Gene and Cell Therapy	Gene Therapy Product	
		Viral	Non Viral
Identity	Surface marker determination DNA fingerprinting; STR analysis Species Morphology Bioassay Biochemical marker	Restriction enzyme mapping PCR/Q-PCR Immunoassay (transgene) DNA sequencing	Restriction enzyme mapping PCR/Q-PCR Immunoassay (transgene) DNA sequencing
Potency	Viability Titer Bioassays include • Colony formation • Functional transgene expression • Secondary effects (i.e., downstream effects)	Functional transgene expression	Functional transgene expression
Purity	Cell viability Transduction efficiency Functional transgene expression (targeted cell) Process contaminants	Residual host cell DNA/RNA Residual host cell protein Process contaminants Helper virus contamination Vector integrity (defective/immature particles)	Plasmid integrity Residual host cell DNA/RNA Residual host cell protein Process contaminants • Solvents, salts, CsCl Vector integrity (defective/immature particles)
Safety	Sterility Mycoplasma Pyrogens, endotoxins Adventitious virus Residual virus/provirus Viral Replication competency Tumorigenicity	Residual host cell DNA (cell and target specific) General safety Sterility Mycoplasma Pyrogens, endotoxins Adventitious virus Viral Replication competency	Sterility Mycoplasma Pyrogens, endotoxins

Gene and Cell Therapy Solutions

Our dedicated departments in support of Identity, Potency, Purity, and Safety testing include:

Analytical – specializing in physicochemical characterization of biologic products and viral and gene therapy vectors

Capabilities: mass spectroscopy, capillary electrophoresis, LC/HPLC chromatography, spectrophotometry

Cell Biology & Immunoassay Services – testing of cell derived products including viral and gene therapy vectors through immunological methods

Capabilities: Flow Cytometry, ELISA/Electrochemiluminescence, Meso Scale Discovery (MSD®) cell-based transduction/infectivity

Microbiology – Microbiology - specializing in sterility and mycoplasma (including avian mycoplasma and spiroplasma)

Capabilities: Microbial Cell Line Characterization (CLC) for microbial-based vector or delivery systems

Molecular Biology – specializing in all nucleic acid based testing and characterization

Capabilities: Sanger & Next generation sequencing, real-time, quantitative PCR (Q-PCR)

Toxicology – specializing in animal model systems

Capabilities: early phase dosing in support of biodistribution and tumorigenicity studies

Virology – General virology, retrovirology, *in vivo* and *in vitro* virology departments supporting all safety testing including replication competency

Gene and Cell Therapy Testing – A Selection of Services Offered

Cell banking services: Experience in manufacturing cell banks supporting the manufacture of virus – e.g., HEK293, PER.C6®, HeLa, Vero, Sf9/21, A549, MRC-5, WI-38 and Vector producing cell lines (VPC)

Copy number by Quantitative Polymerase Chain Reaction (Q-PCR): Allows the quantitative determination of the copy number of a nucleic acid target molecule. Calculation of the copy number is achieved by comparing the standard curve generated by known numbers of target molecules with unknown samples. Q-PCR assays are often employed as identification assays.

E. coli residual DNA/RNA: Bacteria represent the major source for the bulk preparation of plasmids in support of virus manufacture. Determination of the amount of native bacterial nucleic acids is evaluated and presented per milligram of final product.

Fluorescence in situ hybridization studies (FISH): In situ hybridization is a powerful tool for localizing DNA sequences. The technique is sensitive, allowing sequences a few kilobases long to be routinely detected. The relative copy number distribution can be determined for sequences present at more than one site.

Karyology: Metaphases are examined for chromosome number (modal chromosome number, frequency distribution and ploidy); banding pattern; quantification of abnormalities including chromosome and chromatid gaps and breaks; and verification of species and cell line. Analysis is offered by classical and SKY (fluorescent chromosome "paint") methods.

Plasmid integrity: Determination of the integrity of the plasmid following preparation/purification is evaluated through measurement of the overall percentage of supercoiled DNA relative to open/nicked plasmid.

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Plasmid retention/residual: Determined by propagating a microbial cell line on the appropriate antibiotics selection media and then determining the percentage of total cells plated that survive. Q-PCR based assays designed to detect an antibiotic selection marker(s) via end-point detection can also enhance testing for plasmid retention in bulk product.

Quality by Design (QbD) services: BioReliance can work with you to design and execute customized biosafety testing programs – from testing of input raw materials using our proprietary Massively Parallel Sequencing service (MP-Seq™) to the subsequent design of molecular and analytical solutions supporting downstream applications and final product release.

Residual host cell analysis: Detection and quantification of residual host cell nucleic acids and proteins are performed via molecular biology based methods. If the gene therapy vector is manufactured in human cell lines, a sizing option may be included.

Stability Packages: Customized assay packages evaluating physicochemical properties, identity, and potency of gene therapy vectors following short, medium, and long-term storage (typically up to 5 years).

Vector nucleic acid (DNA, RNA) sequencing: Confirmation of sequence integrity of gene therapy vectors. Sequencing is performed by traditional Sanger (capillary-based) sequencing or by Massively Parallel Sequencing based (MP-Seq™) methods. MP-Seq™ can provide an unparalleled depth of coverage by sequencing millions of bases per run (enough to sequence an entire viral genome).

Virus manufacture: SAFC Carlsbad is a leader in the contract manufacturing of viral vaccines and gene therapy drug products. From pre-clinical development to manufacturing and fill/finish, our state-of-the-art, GMP facility supports therapeutic virus production of Adenovirus, Adeno Associated Virus, Lentivirus, Herpesvirus, Vaccina, Reovirus, Coxsackie, Dengue, Sindbis, Retrovirus, Alphavirus and many other viral products. BioReliance's UK facility also offers viral manufacturing and cell banking services. The UK Manufacturing team has experience in all the standard cell lines and virus types prevalent in the gene therapy industry and are licensed for commercial manufacturing.

Viral replication competency: Determination/detection of viral gene therapy vector replication. Routine and customized assays are offered for all major vector categories.

In support of clinical applications:

DNA fingerprinting – STR analysis: Cell line authentication and/or tracking and cross-contamination tests of patient cell lines can be performed by fragment analysis of amplified hypervariable sequences. The pattern of gel-resolved fragments obtained is unique for each cell line.

Biodistribution: Gene therapy vectors are evaluated in animal model systems whereby extraction of organs and tissues following virus dosing can be monitored. Detection of virus and transgene is then determined via molecular (Q-PCR) or immunological methods.

References and Regulations

1. 10-1327 - United States of America v. Regenerative Sciences, Llc et al
2. USP 36 <1047> Gene Therapy Products/General Information
3. European Pharmacopoeia 5.14 Gene transfer medicinal products for human use
4. PER.C6 is a registered trademark of Crucell Holland B.V.
5. Electrochemiluminescence and Meso Scale Discovery (MSD) are trade marks of Meso Scale Diagnostics LLC.

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