DRAFT

History and Characterization of the Vero Cell Line

A Report prepared by
CDR Rebecca Sheets, Ph.D., USPHS
CBER/OVRR/DVRPA/VVB

for

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Introduction

CBER is requesting expert advice from the Vaccines and Related Biological Products Advisory Committee (VRBPAC) upon the use of Vero cells for the production of viral vaccines. To provide you with information upon which to consider your feedback, confidential information about products under review in Investigational New Drug Applications (INDs) has been provided to you under separate cover. In addition, below you will find a summary about the published history and characterization of Vero cells, as well as a brief description of the tests recommended by CBER for characterization of cell lines used to produce biologicals, as promulgated in the 1993 "Points to Consider in the Characterization of Cell Substrates Used to Produce Biologicals" (referred to herein as the PTC) and the International Conference on Harmonisation (ICH) Q5D document entitled "Guidance on Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products" published in the Federal Register on 9/21/98.

Currently, the Vero cell line is used to produce only one U.S. licensed viral vaccine, inactivated poliovirus vaccine produced by Aventis Pasteur. However, CBER has received proposals either in pre-IND or under IND to use Vero cells to produce many other viral vaccines, including live viral vaccines. With the exception of this one licensed inactivated and purified vaccine, all other licensed vaccines are produced in diploid cell strains (WI-38, MRC-5, FRhL-2), yeast, or primary cells (eggs, primary monkey kidney cells) which are considered "normal." In contrast, the Vero cell line is a continuous cell line, which is aneuploid and will grow indefinitely in culture. At the passage levels used for vaccine manufacture, Vero cells do not form tumors in immunosuppressed rodents. However, these cells are not "normal" diploid cells used to produce other types of licensed viral vaccines. Concerns over use of "normal" or "abnormal" cells are described in literature cited in the attached bibliography and will not be discussed herein. However, the committee may wish to consider these concerns, as similar issues are of current concern regarding continuous cell lines as were considered for human diploid cell strains historically. This is reviewed in two of the articles cited in the bibliography below and provided to the committee.

History

The Vero cell line was derived from the kidney of a normal, adult, African green monkey (Cercopithecus) on March 27, 1962, by Y. Yasumura and Y. Kawakita at the Chiba University in Japan (Nippon Rinsho 21:1209, 1963). The cell line was brought to the Laboratory of Tropical Virology, NIAID, NIH, at passage level 93 from Chiba University by Dr. B. Simizu on June 15, 1964. There is documentation of the types of culture media and the concentration of bovine serum used in the media throughout its history. In addition to its use as a vaccine cell substrate, this cell line has been used extensively for virus replication studies and plaque assays. Vero cells are sensitive to infection with SV-40, SV-5, measles, arboviruses, reoviruses, rubella, simian adenoviruses, polioviruses, influenza viruses, parainfluenza viruses, respiratory syncytial viruses, vaccinia, and others. The cell line was submitted to the ATCC at passage level 113 and

was propagated to passage level 121 at the ATCC to establish a bank of cells for availability. Thus, most vaccine manufacture is performed with cells at passage levels in the 130's or 140's, after further propagation for establishment of manufacturer's master and working cell banks and culture for vaccine production.

Initial characterization of Vero cells as a substrate for vaccine production was performed by the Institut Mérieux, now known as Aventis Pasteur. This company has been licensed in France to manufacture inactivated poliovirus vaccine (IPV), oral (live) poliovirus vaccine, and inactivated rabies vaccine manufactured in Vero cells since the 1980's. The IPV vaccine was licensed in the U.S. in 1990. It is currently the primary IPV used for universal immunization of infants and children in the U.S.

The rationale behind the use of Vero cells rather than primary monkey kidney cells is that these cells can be banked and well characterized, which avoids the issues of lot-by-lot variability and adventitious agent contamination of primary cultures freshly initiated for each production run from the kidneys of wild-caught monkeys. In addition, the continued use of animals is problematic from ethical and economic viewpoints. The rationale behind the use of Vero cells rather than diploid cells is that Vero cells can be more readily adapted for growth in bioreactors on microcarriers and provide consistently higher yields of virus. This allows for greater vaccine purity (less contaminating cell debris), larger lots of vaccine (i.e., greater vaccine availability), and more economic production of vaccine. The issues of yield and adaptability to growth in bioreactors are the main grounds for use of Vero cells provided to CBER by most manufacturers who propose to use them for vaccine production.

Recommended Characterization and Adventitious Agent Testing

Scientists at the Institut Mérieux have published articles about the banking and characterization of Vero cells, which they have performed. This will be summarized below. Much of this testing conforms with the recommendations promulgated by CBER in the PTC or by the ICH in the Q5D document. Specifically, these documents recommend that cell lines used to produce biologicals be banked and the banks be characterized for their growth characteristics, identity, donor history, safety and purity from adventitious agents, and tumorigenicity.

Growth characteristics include passage level, doubling time, morphological appearance, and if transfected to express a foreign gene, stability of the foreign gene expression. Tests for identity include isoenzyme analyses and karyology to confirm the species, the donor gender, and the chromosomal number (diploid, aneuploid) of the cells. These tests confirm that the banked cells are what they are supposed to be and that they have not been grossly (>1%) contaminated with other cells. Donor history documents the age, gender, and species of the donor, the medical history of the donor (which is useful to guide thorough adventitious agent testing of the cells), and history of the isolation and propagation of the cells prior to receipt and banking by the manufacturer.

Adventitious agent testing includes tests to assess bacterial and fungal sterility and freedom from contamination with mycoplasma, *Mycobacterium tuberculosis* (if appropriate), and adventitious viruses. Bacterial and fungal sterility testing is specified in the Code of Federal Regulations (CFR). Mycoplasma testing includes broth and agar cultures for cultivatable mycoplasmas and indicator cell culture for non-cultivatable mycoplasmas. *Mycobacterium tuberculosis* contamination can be detected by culture or in vivo in guinea pigs (6 week test). Adventitious virus testing is described further in the next paragraph. In addition, serum or other bovine components used in the culturing or manufacture of biological products must be certified to come from herds free of the agent which causes bovine spongiform encephalopathies, since testing for this agent is extremely difficult and unvalidated.

Adventitious virus testing is also referred to as testing for extraneous or inapparent viruses. There are multiple tests intended to detect different types of adventitious viruses, including tissue culture or *in vitro* tests, *in vivo* tests, tests for antibody production in rodents to specific rodent viruses, tests for retroviruses, and tests for specific viruses [i.e., polymerase chain reaction (PCR) tests for specific viruses].

Tissue culture or *in vitro* tests are recommended to be performed by exposing culture fluids from the banked cells to monolayer cultures of human diploid cells, monkey kidney cells, and cells of the same species and tissue type as the production cells. Most manufacturers use the production cells themselves to fulfill this latter recommendation. At the end of the observation period, usually two weeks, the cultures are tested for hemadsorption or hemagluttination using red blood cells from various species, including chicken, guinea pigs, and human. If the production cells are capable of propagating the growth of human cytomegalovirus, then the human diploid cell cultures should be observed for at least four weeks. In addition, although not specifically recommended in the PTC, some manufacturers will co-cultivate the production substrate with these various cell lines in a similar test. Also, not specifically recommended, but sometime performed, the production substrate itself will be propagated and tested for hemadsorption or hemagluttination directly.

In vivo tests are recommended to be performed by injecting culture fluids or cell lysates into adult and suckling mice and embryonated hens' eggs. In some cases, testing may also be recommended in guinea pigs, rabbits, or monkeys. Many of the methods for the conduct of these tests were promulgated in the CFR in regulations which have subsequently been revoked to permit greater flexibility in regulatory action (the additional standards for vaccines, 21 CFR 630.35). However, the test methods are still recommended (although no longer required by regulation). The tests in adult and suckling mice involve injection of specific volumes intracerebrally and intraperitoneally with an endpoint of survival of the animals through specific subsequent observation periods. While the test in adult mice is capable of detecting lymphocyctic choriomeningitis virus (LCM), another more specific test is also recommended in the PTC for characterization of rodent cell substrates. The tests in embryonated eggs similarly involve injection of specific volumes into the allantoic fluids or yolk sacs.

Tests for antibody production to rodent viruses involve injecting specific pathogen free rodents (mouse, rat, or hamster) and testing for production of specific antibodies, i.e., seroconversion to specific rodent agents. These tests are recommended for rodent cell substrates, e.g., if Chinese hamster ovary (CHO) are used for biological production, then HAP (hamster antibody production) testing is recommended. Likewise, for a murine monoclonal antibody, MAP testing is recommended.

Tests for retroviruses include transmission electron microscopy (TEM), reverse transcriptase assays, and infectivity assays. TEM is a generic, but relatively insensitive, test capable of detecting gross contamination of cells with viruses or other adventitious agents. Cells producing retroviruses at sufficiently high titer can be observed to have retroviral particles budding from the cell surfaces or present in the inter-cellular spaces. In some cases, manufacturers have performed TEM on cells which have been induced to express retroviruses, although this is not specifically recommended in the current PTC or ICH documents. Reverse transcriptase assays include the conventional assay using polynucleotide templates in the presence of magnesium and, separately, manganese, or PCR-based assays, which are significantly more sensitive, but also capable of detecting the reverse transcriptase activity inherently present in cellular DNA polymerases. Infectivity assays are generally intended to amplify and detect murine retroviruses, since murine cells endogenously express them, or can be induced to express them. However, infectivity assays or co-cultivation assays designed to detect simian or human retroviruses have been employed in the characterization of Vero cells by some manufacturers.

Specific viruses recommended to be tested in the PTC include Epstein Barr virus, cytomegalovirus, hepatitis B and C viruses, papillomaviruses, adenoviruses, and Herpes 6 and 7 viruses. These tests are performed using PCR. Human cell substrates are recommended to be tested for these agents depending on the donor's medical history and the tissue source. Some manufacturers have utilized PCR to screen Vero cells for simian viruses, such as simian immunodeficiency viruses and simian foamy viruses.

Tumorigenicity testing is recommended to be performed on specimens containing 10^7 cells/animal (1993 PTC – 1987 PTC recommended 10^6 cells/animal) in immunocompromised rodents, e.g., nude mice; newborn hamsters, mice, or rats immunosuppressed with antithymocyte serum or globulin; or thymectomized and irradiated mice reconstituted with bone marrow from healthy mice. Alternatively, oncogenicity tests may be performed examining colony formation in soft agarose or growth in organ culture, if shown to be at least as sensitive as *in vivo* testing. Some manufacturers perform both kinds of tests.

Characterization of Vero Cell Line

Scientists at the Institut Mérieux have published articles about the banking and characterization of Vero cells, which they have performed. Attached you will find some of these articles.

Identity testing performed: karyology – Vero cells are aneuploid; isoenzyme analyses, including lactate dehydrogenase, phospho-gluconate dehydrogenase, and glucose phosphate isomerase, indicate Vero cells are from Cercopithecus and differ from Hep-2, WI-38, and MRC-5 cells (human cell strains or lines).

Adventitious agent testing performed: bacterial and fungal sterility; mycoplasma; tests in suckling mice, adult mice, rabbits, guinea pigs, and embryonated eggs; co-cultures of whole and lysed cells with human and simian cells; electron microscopy on induced or uninduced cells; reverse transcriptase assay; Southern blotting for SIV_{agm}, HIV-1, and Mason-Pfizer monkey virus (MPMV) on DNA extracts of Vero cells; Northern blotting of RNA extracted from Vero cells for MPMV; and radio-immunoprecipitation assay for MPMV, SIV_{agm}, and HIV-1. In addition, Vero cells were spiked with a high titer of SIV_{agm} and did not propagate growth of this virus.

Tumorigenicity testing was performed in hamsters, immunosuppressed newborn rats, and nude mice at several passage levels of Vero cells, including 142 (at or beyond vaccine production passage level), 161, 169, 191, and 211 and at 10^6 or 10^7 cells/animal. Progressively growing nodules and lung and lymph node metastases were observed only at passage levels 191 and 211 with either inoculum.

Oncogenicity testing was stated to have been performed by growth in soft agar (although results of this test are not clearly described in the articles by the scientists at the Insitut Mérieux). However, in testing performed by FDA (Petricciani et al.) at passages between 127-140 and 162-265, Vero cells were reported to form colonies in soft agar and tumors in organ cultures (tumors were not formed in nude mice at passages 127-140, but were at passages 162-265).

Characterization by any sponsor

A table listing a composite of adventitious agent testing by any sponsor under U.S. IND is attached. You will note that virtually every test recommended in the PTC has been applied to Vero cells by some sponsor. In addition, specific testing for several other agents not described in the PTC have been performed by one or more sponsors under U.S. IND. A list of abbreviations and definitions used in the table are appended below.

Summary

Vero cells have been extensively tested and shown to be free from adventitious agents for which there are currently available tests and to the limit of sensitivity of those tests. However, this cell line is an aneuploid cell line which does not form tumors at the passage levels used to produce vaccines, but develops tumorigenic potential at higher passage levels. Globally, millions of people have received inactivated poliovirus vaccine, inactivated rabies vaccine, and oral (live) poliovirus vaccine manufactured in Vero cells by Aventis Pasteur. The most widely used inactivated poliovirus vaccine (IPV) used in the U.S. is that made by Aventis Pasteur in Vero cells. IPV is recommended for universal immunization of infants. Numerous investigational products are being produced or have been proposed to be produced in Vero cells. Herein has been provided a summary of the published information about the characterization of Vero cells used to produce viral vaccines, as well as brief descriptions of the tests used to characterize cell substrates.

Bibliography of Suggested Reading and Source Documents

"Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (CBER, 1993)

"International Conference on Harmonisation; Guidance on Quality of Biotechnological/Biological Products: Deriviation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products;" Federal Register, 63(182):50244-49; Sept. 21, 1998

"International Conference on Harmonisation; Guidance on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin;" Federal Register, 63(185):51074-84; Sept. 24, 1998

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Vincent-Falquet, J.C., Peyron, L., Souvras, M., Moulin, J.C., Tektoff, J., and Patet, J.; "Qualification of Working Cell Banks for the Vero Cell Line to Produce Licensed Human Vaccines;" Developments in Biological Standardization, 70:153-56; 1989

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Fletcher, M.A., Hessel, L., and Plotkin, S.A.; "Human Diploid Cell Strains (HDCS) Viral Vaccines;" Developments in Biological Standardization, 93:97-107; 1998

Meulien, P.; "DNA Issues;" Developments in Biological Standardization, 93:57-60; 1998

Lupker, J.H.; "Residual Host Cell Protein from Continuous Cell Lines – Effect on the Safety of Protein Pharmaceuticals;" Developments in Biological Standardization, 93:61-64; 1998

Abbreviations and Definitions Used in Table of Adventitious Agent Testing

M or MCB - Master Cell Bank – this represents the lowest passage level of Vero cells which the manufacturer has banked for use for vaccine production

W or WCB or MWCB – Manufacturer's Working Cell Bank – this represents the bank of Vero cells from which the manufacturer will take vials of frozen cells to initiate cultures from which vaccine will be produced

P – Production Cells – usually this refers to control cells, which are cells split off prior to infection with the vaccine virus, and carried through culture in the same way that the infected cells are. However, this can refer to actual production cells used to produce vaccine.

E or EOP or EOPC – end-of-production-cells – usually this refers to cells that have been grown from a vial of MCB or WCB to a passage level equivalent to or several passages beyond (e.g., 10) the highest passage level that would be used during vaccine production

ND – not done – tests were not performed on the Vero cell banks (**when these tests were performed on other raw materials, e.g., serum or trypsin, or on the viral seeds or viral harvests, but were not specifically performed on the Vero cell banks, they are listed as not done – this does not indicate that the product has not been suitably tested for appropriate agents**).

TC – tissue culture

cult.; non-cult. – cultivatable or non-cultivatable Mycoplasma – tests for these organisms are done by culture (for cultivatable Mycoplasma) on broth or agar or through use of indicator cells (for non-cultivatable Mycoplasma, e.g., Mycoplasma hyorrhinis)

tests using animals or eggs were described in the revoked regulations and are still applied – these include tests in suckling mice, adult mice, guinea pigs, rabbits, monkeys, and embyronated eggs

TEM – transmission electron microscopy, an insensitive, but general method for visualizing adventitious contaminants in cell cultures

RT testing – reverse transcriptase tests may be the conventional test using polynucleotide templates and assessing reverse transcription in the presence of manganese or magnesium or may be a polymerase chain reaction (PCR)-based test

MAP, RAP, HAP – murine antibody production, rat antibody production, hamster antibody production – tests for common rodent adventitious agents by exposure of specific-pathogen-free (SPF) rodents to test article and assessment of antibody

responses – if an adventitious agent is present, the rodents will seroconvert to that agent

PCR – polymerase chain reaction assay

IBR, PIV-3, BVDV, BAV3, BPV, REO3, rabies – agents which infect bovines and can be introduced from bovine serum into cultures

LCM – lymphocytic choriomeningitis virus – this murine contaminant can be detected by the *in vivo* test in adult mice, but a more specific test for this virus can also be performed

CMV – cytomegalovirus

HAV, HBV, HCV – hepatitis viruses

EBV, HHV-6, -7, HSV-1, -2 – human herpes viruses

SIV, SFV, STLV – simian retroviruses

HIV-1, -2 – human immunodeficiency virus

Cells used in tissue culture infectivity or retrovirus infectivity tests:

MRC-5 – human diploid cell strain

WI-38 – human diploid cell strain

6C2 – erythroleukemic fowl cells

FL-5000 – whole human embyro fibroblast cells

HeLa – human cervical carcinoma cell line

Hep2 – cell line which was contaminated with and replaced by HeLa cells

LLC-MK2 – rhesus monkey kidney cell line

BSC-1 – Cercopithecus (African green) monkey kidney cell line

A549 – human lung carcinoma cell line

MDBK – Madin-Darby bovine kidney cell line

RK-13 – rabbit kidney cell line

Mus dunni – murine cells used to detect murine retroviruses

PBL – peripheral blood lymphocytes

CEM – human T-lymphoblastoid cell line

Cf2Th – canine thymus cell line

Molt 4 Clone 8 – human T-lymphoblastic cell line

Composite of All Adventious Agent Testing on Vero Cells under IND by Any Sponsor

Test	Stage	Comments
Sterility (Bact., Fungi)	M, W, E, P	14- & 21-day
Mycoplasma	M, W, E	cult. & non-cult.
TC test	M, W, E, P	*
suckling mice	M, W, E, P	
adult mice	M, W, E, P	
guinea pigs	M, W, E, P	
rabbits	M, W, E, P	
embryonated eggs	M, W, E, P	
monkeys	ND	
TEM	M, W, E	
RT testing	M, W, E	convent. & PBRT
Retrovirus Infectivity	M, W, E	**
MAP, RAP, HAP	E	
porcine parvovirus	M, W, E	
IBR, PIV-3, BVDV, BAV, BPV	M, W, E	REO3 & rabies
LCM	M, W, E, P	
EBV	M, E	
human CMV	M	
HAV, HBV, HCV	E	
papilloma	M, E	
adenovirus	ND	
HHV-6, HHV-7	M, E	
Mycobacterium	M, W, E, P	Culture, g. pigs

M=Master
W=Working
P=Production
E=EOP
cult. = cultivatable
ND=not done

* WI-38, MRC-5, Vero, primary rabbit kidney, primary Cercopithecus monkey kidney cells primary human amnion, FL-5000, HeLa, chick embryo cells, RBK, Hep-2, LLC-MK2, BSC-1, rhesus monkey kidney cells

** 6C2 cells, mink S+L-, XC plaque assay, Vero (Simian Foamy Virus PCR read-out), Molt 4 clone 8 (SIV PCR read-out), Mus dunni, A549, human PBL (HIV p24 read-out), CEM, Cf2Th

Additional Testing (not recommended in PTC)

Simian Immuno. Virus	E	PCR, infectivity
Simian T-Lympho. Virus	E	PCR
HSV-1, -2	M	PCR
Adeno-associated virus	M	Southern blot
Mason-Pfizer monkey virus	E	Southern blot
SV40	E	PCR
Simian CMV	E	PCR
Bovine polyoma virus	E	PCR
HIV-1, -2	E	PCR