THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR: Dr. Cindy Hutnik
DEPARTMENT: ophthalmology
ADDRESS: 268 Grosvenor St.
PHONE NUMBER: 519-646-6272
EMERGENCY PHONE NUMBER(S):
EMAIL: cindy.hutnik@sjhc.london.on.ca

Location of experimental work to be carried out: Building(s) F Room(s) B-16/B-13

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Oxidative Stress in the pathogenesis of AMD - The Relationship between Oxidative Stress and Complement 43

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Sarah Harasym	Sharasym@uwo.ca	July 15/10
Christopher Byrne	cbyrne23@uwo.ca	June 18/10
Hong Liu	Hong.Liu@sjhc.london.on.ca	

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Decontamination and Waste Disposal Procedure in Room FB113

Liquids
Liquid Wastes, those in which containment level 1 & 2 agents have been cultured, require sterilization or disinfection to inactive agent before disposal to the sewage system.

Mix 1/10 disinfectant solution (sodium hypochlorite), or 10% bleach, with liquid wastes for 20 to 30 minutes before disposal.

Solids
Disposable non-sharp items (empty plastic culture dishes, flasks and tubes, etc.) that are contaminated with biological agents should go to biohazardous garbage.

Disposable sharps (Pasteur pipettes, scalpels) that are contaminated with biological agents are collected in the special sharps containers for infectious wastes.

Reusable items such as glassware are first chemically disinfected by detergent, and then are autoclaved in steel containers.

PLEASE SEE ATTACHED FILE FOR BIOLOGICAL AGENT DESCRIPTION AND STORAGE

Please include a one page research summary or teaching protocol
VEGF PROJECT SUMMARY

Purpose: To determine the effect of connexin43 (Cx43) expression and gap junctional intercellular communication (GJIC) on the expression and secretion of vascular endothelial growth factor (VEGF) from the retinal pigment epithelium under normal cell culture and oxidative stress conditions.

Methods: Stable cell lines of ARPE-19 were produced in which Cx43 was either over-expressed, down-regulated by targeted shRNA, or functionally inhibited by co-expression of a disease-linked dominant-negative mutant (G21R). Pharmacologic blockade of GJIC was accomplished with flufenamic acid. Oxidant challenge was performed with *tert*-butyl hydroperoxide (tBH). VEGF gene expression and secretion were assessed by real-time PCR and ELISA, respectively.

Results: Over-expression of Cx43 in ARPE-19 cells reduced both gene expression and secretion of VEGF. Down-regulation of Cx43 increased gene expression and secretion of VEGF. Increased secretion of VEGF was also observed in ARPE-19 cells expressing a dominant-negative mutant of Cx43 and when GJIC was blocked. Over-expression of Cx43 reduced tBH-induced secretion of VEGF from ARPE-19 cells.

Conclusions: Down-regulation and functional inhibition of Cx43 in ARPE-19 cells increases VEGF secretion under normal cell culture conditions, and expression of Cx43 protects against oxidative stress-induced VEGF secretion.

Introduction

Age-related macular degeneration (ARMD) is a prevalent eye disease in which choroidal neovascularization (CNV) contributes to significant visual loss. Vascular endothelial growth factor (VEGF) is well-recognized for its critical role in the progression of neovascular ARMD (Rakic et al., 2003). Indeed, VEGF is now a key therapeutic target for treatment of this condition (Pieramici et al., 2006). Recent reports suggest that the retinal pigment epithelium (RPE) may be an important source of VEGF (*Witmer et al., 2003)*. Under normal conditions, the RPE produces a basal level of VEGF that interacts with receptors on nearby choroidal endothelial cells to promote normal endothelial cell function (*Saint-Geniez et al., 2006*). However, elevation of VEGF beyond basal levels may contribute to neovascularisation in ARMD. High levels of VEGF have been documented in RPE cells of surgically excised retinas of patients with ARMD (*Lopez et al., 1996; Ikeda et al., 2006*), and over-expression of VEGF by the RPE has been shown experimentally to cause CNV (*Baffi et al., 2000*). Consequently, much attention has been given to the identification of factors that modulate the production of VEGF by the RPE.

Disruption of the RPE monolayer is thought to be an early event in the pathogenesis of ARMD and a recent study has shown that disruption of adherens intercellular junctions enhances RPE secretion of VEGF (*Ikeda et al., 2006*). Like adherens junctions, gap junctions and their basic connexin (Cx) units are key contributors to the maintenance of membrane monolayer integrity. Connexins have been shown to regulate VEGF expression in other cell models (*Shao et al., 2005; McLachlan et al., 2006; Laws et al., 2008*), and the purpose of this study was to determine whether a regulatory influence of Cx43 on VEGF also exists in a human RPE cell line. Of the 21 members of the human Cx family, Cx43 was chosen for this study due to its wide expression across tissue types and its presence in RPE cells (*Malfait et al., 2001*). Further, we have previously demonstrated that expression of Cx43 in RPE cells is protective against oxidative stress-induced cell death (*Hutnik et al., 2008*). Interestingly, oxidative stress is another factor implicated in early ARMD pathogenesis (*Cai et al., 2000*) and in stimulation of VEGF secretion from the RPE (*Kannan et al., 2006; Ayalasomayajula and Kompella, 2002*). Given our hypothesis that Cx43 expression regulates VEGF production from the RPE, we questioned whether it would also exert an effect on oxidative stress-induced VEGF secretion.

The current study was conducted to investigate the link between Cx43 expression and VEGF production in a human retinal pigment epithelial cell line, and to determine the effect of Cx43 on oxidative stress-induced secretion of VEGF from these cells. As the current use of VEGF inhibitors represents a significant, yet imperfect, intervention for ARMD, it is our hope that understanding upstream events leading to VEGF release by the RPE will open avenues to more targeted and effective therapies for this prevalent and burdening disease.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES ⊗ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ⊗ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ⊗ YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	⊗ Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	X O Yes O No	Trabecular Meshwork Cell	ScienCell
Rodent	O Yes O No	Retinal pigment epithelium cell	ATCC
Non-human primate	O Yes O No	corneal epithelium cell	
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES X NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES X NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below ⊗NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ⊗NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES ⊗NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES Ø No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES Ø NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES ⊗ NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ ⊗ NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ✓ O 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

✓ YES, permit # if on-campus B10 - LHRI - 0063
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: Sept 22/10

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

_____
_____

See E-mail

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: Oct 12/ 2010

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

ScienCell™
Research Laboratories

Human Trabecular Meshwork Cells (HTMC)

Catalog Number: 6590

Cell Specification

Trabecular meshwork cells (TMC) play an active role in the aqueous outflow mechanism. Specific receptors for neurotransmitters and neuropeptides, including epinephrine, acetylcholine, and neuropeptide Y, have been identified in TMC. In addition, a long list of vsoactive peptides and growth factors trigger intracellular signaling mechanisms in TM cells [1]. TMC synthesize a great variety of extracellular matrix proteins as well as metelloproteinases. TMC culture has provided an invaluable tool to characterize pharmacological properties of TMC [2].

TMC from ScienCell Research Laboratories are isolated from juxtacanalicular and corneoscleral region of human eye. TMC are cryopreserved on primary culture and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HTMC are characterized by immunofluorescent method with antibodies to alpha-smooth muscle actin and fibronectin. TMC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. TMC are guaranteed to further expand for 15 population doublings in the conditions specified by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Fibroblast Medium (FM, Cat. No. 2301) for the culturing of TMC *in vitro*.

Product Use

TMC are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

Shipping

Dry ice.

Reference

[1]. Artur Llobet, Xavier Gasull and Arcadi Gual (2003) Understanding trabecular meshwork physiology: A key to the control of intraocular pressure? *News Physiol Sci* 18:205-209.

[2] Jun Ueda, Kelly K. Wentz–Hunter, E. Lillian Cheng, Takeo Fukuchi, Haruki Abe, and Beatrice Y.J.T. Yue (2000) Ultrastructural Localization of Myocilin in Human Trabecular Meshwork Cells and Tissues. *J Histochem Cytochem* 48:1321–1329.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the ordering:

1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-75 flask is recommended) and leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm^2 is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that trabecular meshwork cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be doubled after two to three days in culture.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks.
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).

ATCC®

Product Information Sheet for CRL-2302

Cell Line Designation: ARPE-19
ATCC® Catalog No. CRL-2302

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: eye; retinal pigmented epithelium; retina
Age: 19 years
Gender: male
Morphology: epithelial
Growth properties: adherent
DNA profile (STR analysis)
Amelogenin: X,Y
CSF1PO: 11
D13S317: 11,12
D16S539: 9,11
D5S818: 13
D7S820: 9,11
TH01: 6,9.3
TPOX: 9,11
vWA: 16,19
Karyotype: diploid
AntigenExp: RPE-specific markers CRALBP and RPE-65
Depositors: L.M. Hjelmeland
Comments: ARPE-19 is a spontaneously arising retinal pigment epithelia (RPE) cell line derived in 1986 by Amy Aotaki-Keen from the normal eyes of a 19-year-old male who died from head trauma in a motor vehicle accident. The cells were subjected to selective trypsinization for the first four passages to remove superficial cells before passaging the cuboidal basal layer.

By passage 5, the cultures appeared to be rapidly growing RPE cells, which would form cobblestone monolayers, which pigmented after several months in culture.

The line was established in a 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 medium with HEPES buffer containing 20% fetal bovine serum, 56 mM final concentration sodium bicarbonate and 2 mM L-glutamine and incubated at 37C in 10% CO2.

These cells form stable monolayers, which exhibit morphological and functional polarity. ARPE-19 expresses the RPE-specific markers CRALBP and RPE-65. The cells exhibit morphological polarization when plated on laminin-coated Transwell-COL filters in medium with a low serum concentration. They form tight-junctions with transepithelial resistance of monolayers reaching a maximum of 50 to 100 ohms/cm2 after 4 weeks of culture.

The cells are diploid and can be carried for over 30 passages. Progeny were found to undergo an additional 48 population doublings in longevity trials performed during characterization at ATCC.

Related Product: ATCC CRL-2502™ is derived from CRL-2302™

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as .the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to7 minutes.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

in polarized epithelial cells. J. Virol. 70: 8402-8410, 1996 PubMed: 8970961

Holtkamp GM et al. **Polarized secretion of IL-6 and IL-8 by human retinal pigment epithelial cells.** Clin. Exp. Immunol. 112: 34-43, 1998 PubMed: 9566787

Finnemann SC et al. **Phagocytosis of rod outer segments by retinal pigment epithelial cells requires alpha(v)beta5 integrin for binding but not for internalization.** Proc. Natl. Acad. Sci. USA 94: 12932-12937, 1197 PubMed: 9371778

Handa JT et al. **The advanced glycation endproduct pentosidine induces the expression of PDGF-B in human retinal pigment epithelial cells.** Exp. Eye Res. 66: 411-419, 1998 PubMed: 9593635

Dunn KC et al. **Use of the ARPE-19 cell line as a model of RPE polarity: basolateral secretion of FGF5. Invest.** Ophthalmol. Vis. Sci. 39: 2744-2749, 1998 PubMed: 9856785

Tugizov S et al. **An acidic cluster in the cytosolic domain of human cytomegalovirus glycoprotein B is a signal for endocytosis from the plasma membrane.** J. Virol. 73: 8677-8688, 1999 PubMed: 10482621

Orten DJ et al. **Analysis of DNA elements that modulate myosin VIIA expression in humans.** Hum. Mutat. 14: 354, 1999 PubMed: 10502787

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02/09

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Cell Line Designation:
10.014 pRSV-T
ATCC® Catalog No. CRL-11515™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: eye; cornea; epithelial; transfected with a plasmid containing the SV40 early region
Morphology: epithelial
Growth properties: adherent
Depositors: Gillette Medical Evaluation Laboratories; C.R. Kahn
Comments: A primary culture of normal corneal epithelium was immortalized by transfection with plasmid pRSV-T using lipofectamine overnight at 37C. pRSV-T contains the SV40 early region genes and the Rous Sarcoma virus long terminal repeat.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

Cell lines and hybridomas deposited for patent purposes are not always screened for contamination, antibody production or characterized by the ATCC. Release of a culture, the use of which may be claimed in a patent, from the ATCC during the effective term of any such patent is not meant to carry with it, and does not grant any license, express or implied, under any patent, or the right to use a culture in any process described in a patent.

The above culture was deposited in the ATCC in connection with a patent application. Copies of U.S. Patents may be obtained from the Commissioner of Patents, U.S. Patent and Trademark Office, Box 9, Washington, D.C. 20231.
This material is cited in a U.S and/or other Patent and may not be used to infringe the patent claims.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

The flasks used must be precoated with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a 15 ml centrifuge tube and dilute with the recommended complete culture medium.

4. Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh medium at the dilution ratio recommended in the specific batch information on a pre-coated flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15*

minutes to allow the medium to reach its normal pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to **precoated** 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

The flasks used should be **precoated** with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin .
Subculture when 80% confluent.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-53mM EDTA solution (GIBCO cat# 25300-054).

3. Add 4.0 to 5.0 ml of Trypsin-EDTA solution to flask and incubate at 37°C for up to three minutes to facilitate dispersal. Observe cells under an inverted microscope. Hit or shake the flask if some cells remain attached.

4. Add fresh medium and aspirate cells by gently pipetting.

5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to10 minutes.

6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new **coated** culture vessels.
Subcultivation ratio: 1:3

7. Place culture vessels in incubators at 37°C. Cells will not be well attached for the first 24 hours after subculture.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Every 2 to 3 days.

Flask Coating

1. Prepare a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin (BSA) dissolved in culture medium. Store pre-prepared Coating Solution at 4°C in cold room for up to 3 months.
2. For a growth area of 75 cm^2, add 4.5 ml of the fibronectin/collagen/BSA solution and rock gently to coat the entire surface.
3. Incubate the freshly coated vessel(s) in a 37°C incubator overnight (it is preferable to use tissue culture vessels with tightened, plug-seal caps to prevent evaporation during the coating process).
4. Store coated flasks with solution at room temperature, light protected, up to 1 month. Suction off solution before plating cells.

Complete Growth Medium

These cells are grown in Keratinocyte-Serum Free Medium (Gibco 17005-042)
Supplemented with 2 frozen additives included (from GIBCO)

1) 0.05 mg/ml bovine pituitary extract (BPE)
2) 5 ng/ml epidermal growth factor (EGF)

and 500 ng/ml hydrocortisone and 0.005mg/ml Bovine insulin not supplied by GIBCO.
Note: Do not filter complete medium
This medium is formulated for use with a 5% CO_2 in air atmosphere.

Cryoprotectant Medium.

The cells are frozen in culture medium, 10% fetal bovine serum and 5% DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

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Additional Information

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References

(additional references may be available in the catalog at www.atcc.org)

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Product Information Sheet for CRL-11515

Lot number: 58780937

Designation: 10.014 pRSV-T Description: Cornea
Total Cells/mL: 1.8 x 10(6)
Expected Viability: 87.3% to 91.4%
Ampule Passage No.: 23
Population Doubling (PDL): N/A
Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)
Volume/Ampule: 1 mL
Date Frozen: 09/08/09

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 80% confluence in 1 day.

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-------- Original Message --------
Subject: Re: Biological Agents Registry Form (Hutnik)
Date: Wed, 27 Oct 2010 13:09:47 -0400
From: Hong Liu <Hong.Liu@sjhc.london.on.ca>
To: Cindy Hutnik <Cindy.Hutnik@sjhc.london.on.ca>, Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

1. Tables2.2/2.3 - our lab do not use rodent of NHP cells.
2. please outline what you would do in case of an accidental exposure: make sure first aid is given immediately complete and give to the worker the treatment. Provide transportation to a hospital if necessary and report to workplace safety (WSIB) within three days if the worker needs more than first aid treatment.

Thanks.

Hong