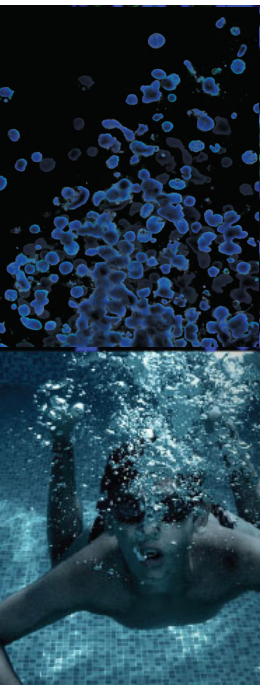
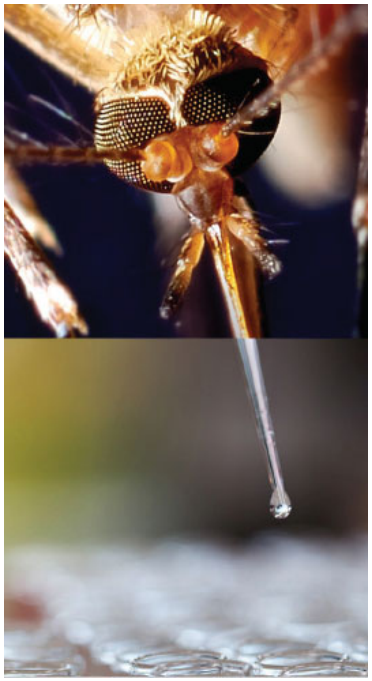
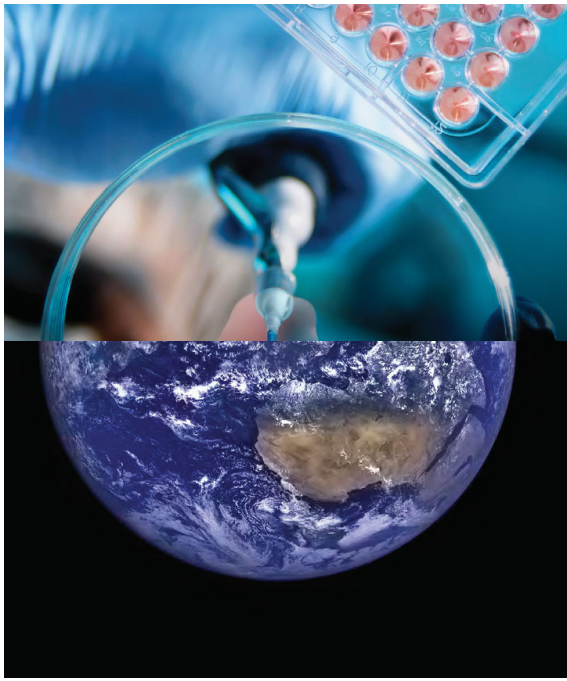
Taming Uncertainty: Reducing Animal By-Products in Cell Biology Workflows

Tiffany Cato, MS
Manager, Lab Operations - Cell Biology, ATCC







Credible Leads to Incredible™

About ATCC®

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents
- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees

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Agenda

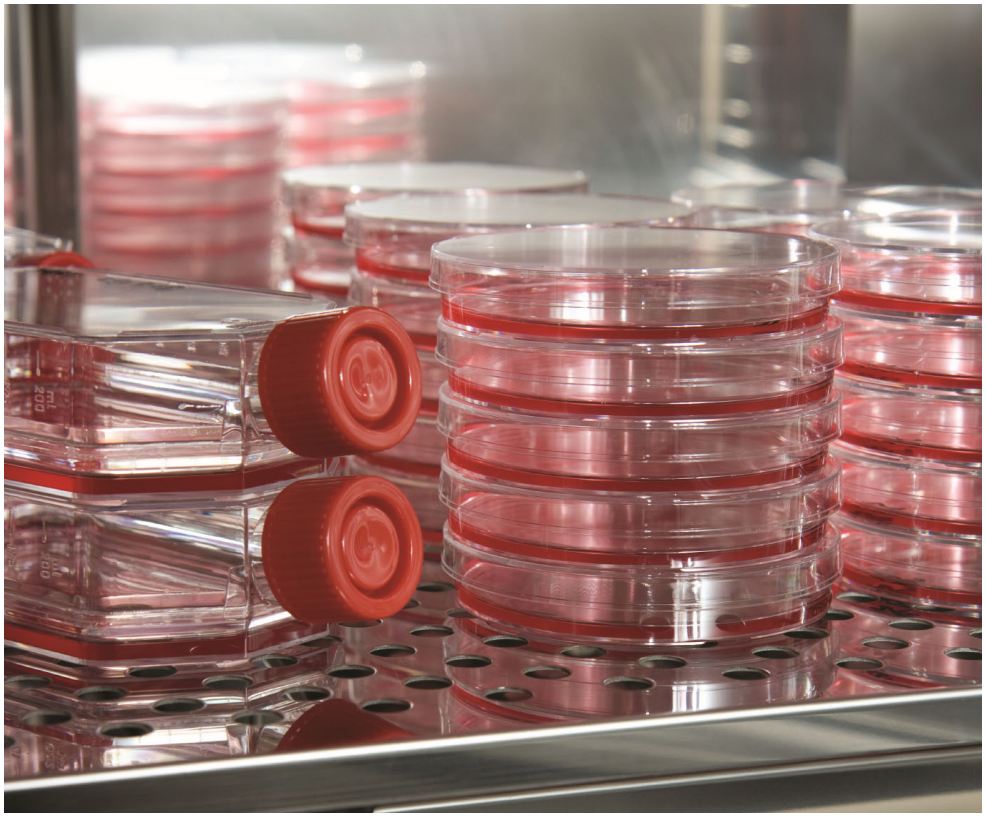
- Cell culture media selection
  - Critical attributes
  - Animal by-product considerations
- ATCC® animal by-product free reagent offerings
- Development of animal by-product free McCoy's 5A

Cell culture media selection

Points to consider before developing your cell biology workflow

Critical attributes to consider when selecting cell culture media:

- Does the media provide the appropriate growth factors and nutrients for the cell type?
- Is the serum well characterized and sourced from Australia, USA, or New Zealand?



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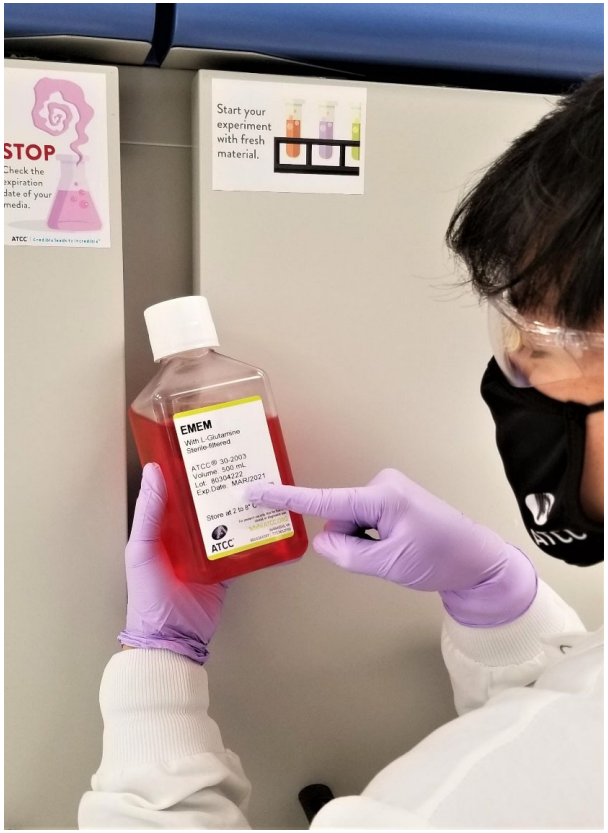
Cell culture media selection

What basal medium to select for your cells?



- For best results, start cell cultures in the same medium used and distributed by ATCC® (listed on the Product Sheet).
- **Eagle's Minimum Essential Medium (EMEM; ATCC® 30-2003™)**
  - Early formula for mouse L cells
  - Amino acid composition to match mammalian cells
- **Dulbecco's Modified Eagle's Medium (DMEM; ATCC® 30-2002™)**
  - Twice the amino acid concentration as EMEM, four times the vitamin concentration
- **Iscove's Modified Dulbecco's Medium (IMDM; ATCC® 30-2005™)**
  - Additional amino acids as DMEM, suited for lymphocytes and hybridomas
- **Kaighn's Modification of Ham's F-12 (ATCC® 30-2004™)**
  - Designed for primary cells with or without serum

Cell culture media selection

Basal media selection

- **DMEM/F12 Medium (ATCC® 30-2006™)**
  - Mixture of DMEM and Ham's F-12, used with a wide range of cell types
  - Proliferation assays
- **McCoy's 5A (ATCC® 30-2007™)**
  - Modified EMEM with additional amino acids and Bacto® peptone.
  - Hepatoma cells and primary cells
- **RPMI-1640 (ATCC® 30-2001™)**
  - Ideal for lymphocytes
- **Leibovitz's L-15 (ATCC® 30-2008™)**
  - For use without CO_2
- **Primary Cells – Primary Cell Basal Media and Growth Kits**
  - Primary cells require their own specially formulated media, specific to each cell type

Cell culture media selection

Points to consider before developing your cell biology workflow

Primary cells require complete culture media, which consists of a basal medium supplemented with appropriate growth factors and cytokines.

Cell Types	Basal medium	Growth Kits
Endothelial	▪ Vascular Cell Basal Medium (ATCC® PCS-100-030™)	▪ Endothelial Cell Growth Kit-BBE (ATCC® PCS-100-040™) ▪ Endothelial Cell Growth Kit-VEGF (ATCC® PCS-100-041™) ▪ Microvascular Endothelial Cell Growth Kit-BBE (ATCC® PCS-110-040™) ▪ Microvascular Endothelial Cell Growth Kit-VEGF (ATCC® PCS-110-041™)
Smooth Muscle	▪ Vascular Cell Basal Medium (ATCC® PCS-100-030™)	▪ Vascular Smooth Muscle Cell Growth Kit (ATCC® PCS-100-042™)
Epithelial	▪ Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030™) ▪ Renal Epithelial Cell Basal Medium (ATCC® PCS-400-030™) ▪ Prostate Epithelial Cell Basal Medium (ATCC® PCS-440-030™) ▪ Bladder Epithelial Basal Medium (ATCC® PCS-420-032™) ▪ Vaginal Epithelial Basal Medium (ATCC® PCS-480-030™) ▪ Cervical Epithelial Cell Basal Medium (ATCC® PCS-480-032™) ▪ Mammary Epithelial Cell Basal Medium (ATCC® PCS-600-030™) ▪ Corneal Epithelial Cell Basal Medium (ATCC® PCS-700-030™)	▪ Bronchial Epithelial Cell Growth Kit (ATCC® PCS-300-040™) ▪ Renal Epithelial Cell Growth Kit (ATCC® PCS-400-040™) ▪ Prostate Epithelial Cell Growth Kit (ATCC® PCS-440-040™) ▪ Bladder Epithelial Growth Kit (ATCC® PCS-420-042™) ▪ Vaginal Epithelial Growth Kit (ATCC® PCS-480-040™) ▪ Cervical Epithelial Cell Growth Kit (ATCC® PCS-480-042™) ▪ Mammary Epithelial Cell Growth Kit (ATCC® PCS-600-040™) ▪ Corneal Epithelial Cell Growth Kit (ATCC® PCS-700-040™)

Cell culture media selection

Points to consider before developing your cell biology workflow

Cell Types	Basal medium	Growth Kits
Fibroblast	▪ Fibroblast Basal Medium (ATCC® PCS-201-030™)	▪ Fibroblast Growth Kit - Serum-free (ATCC® PCS-201-040™) ▪ Fibroblast Growth Kit - Low Serum (ATCC® PCS-201-041™)
Keratinocyte	▪ Dermal Cell Basal Medium (ATCC® PCS-200-030™)	▪ Keratinocyte Growth Kit (ATCC® PCS-200-040™)
Melanocyte	▪ Dermal Cell Basal Medium (ATCC® PCS-200-030™)	▪ Melanocyte Growth Kit (ATCC® PCS-200-041™) ▪ Adult Melanocyte Growth Kit (ATCC® PCS-200-042™)
Mesenchymal Stem Cell and Differentiation Solutions	▪ Mesenchymal Stem Cell Basal Medium (ATCC® PCS-500-030™)	▪ Mesenchymal Stem Cell Growth Kit-Low Serum (ATCC® PCS-500-040™) ▪ Adipocyte Differentiation Toolkit for Adipose-Derived MSCs and Preadipocytes (ATCC® PCS-500-050™) ▪ Osteocyte Differentiation Tool (ATCC® PCS-500-052™) ▪ Chondrocyte Differentiation Tool (ATCC® PCS-500-051™)

Cell culture media selection

Animal by-product considerations

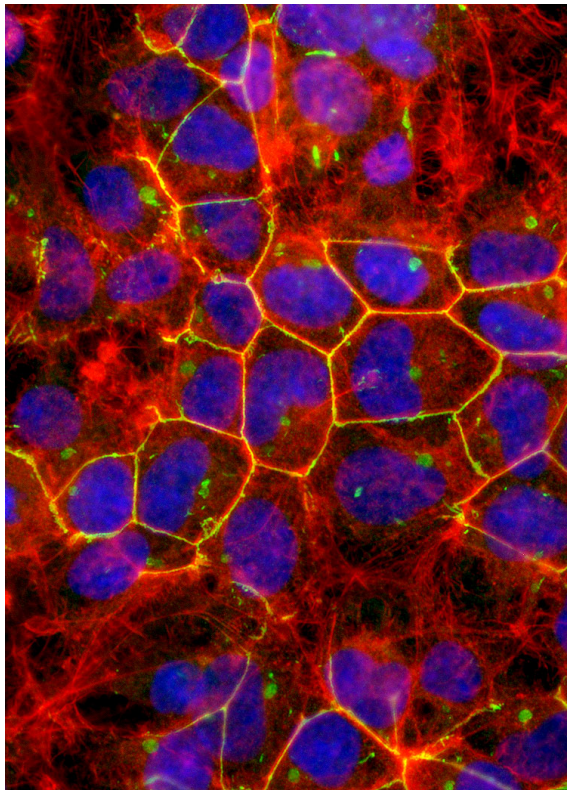
- What are adventitious agents?
- Common sources of adventitious agent contamination:
  - Cell lines with endogenous adventitious agents
  - Animal by-products in cell culture
  - Environment
  - Direct use of animals in bioprocesses
- How to minimize risk?
  - Use well-characterized cell lines from reputable sources
  - In-process and cell bank testing for contamination
  - When animal by-products cannot be avoided, use sera and reagents that are tested according to 9 CFR113.53
  - Use animal by-product free reagents whenever possible as early in development as possible

ATCC

Cell line characterization

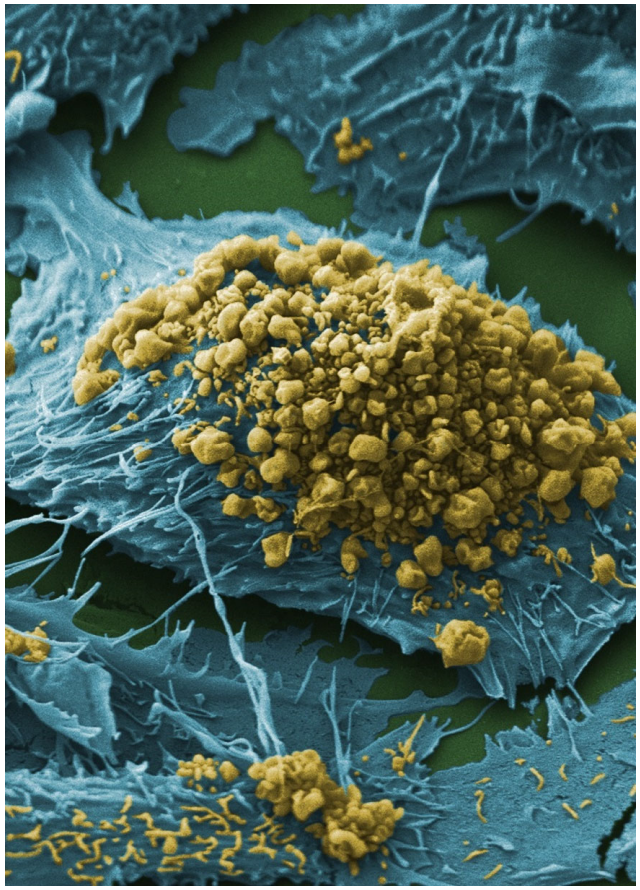
Know your cells

- ATCC® STR Profiling (Human and Mouse) (ATCC Catalog: 135-XV ™)
  - Ensures your cells are what you think they are
  - STR profile of your cell line
  - Comparison of your cells against ATCC® STR Profile database at www.ATCC.org/str
  - Electropherograms supporting the allele calls at each locus
  - Comprehensive interpretation of results
- Cytochrome oxidase 1 (CO1) testing
  - Ensures your cells are what you think they are
  - Determination of species
- Bacterial contamination testing
  - All ATCC® cell lines are tested for aerobic and anerobic bacterial contamination
- Virus testing
  - ATCC® cell lines are tested for HIV, HEPB, HPV, EBV, and CMV



Mycoplasma contamination

Why it matters?



- Common bacterial contaminant
- Class Mollicutes
- No cell wall, simple plasma membrane
- Small cell size, cannot be visualized with microscopy
- Results in a number of harmful effects
- Chromosomal aberrations
- Disruption of nucleic acid synthesis
- Changes in membrane antigenicity
- Inhibition of cell proliferation and metabolism
- Decreased transfection rates
- Changes in gene expression profiles
- Affects virus production
- Cell death

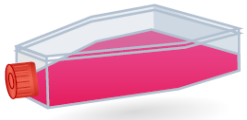
Mycoplasma testing

- When to test for mycoplasma?
  - When you receive a cell line into the laboratory
  - After 10 passages
  - After preparing a cell bank
  - When in doubt
- **Try our detection kit** - ATCC® 30-1012K™
  - Need a quick and sensitive way to detect mycoplasma in your lab? Try our Universal Mycoplasma Detection Kit. We provide the components and protocols you need to detect over 60 species of mycoplasma.
- **Send us your samples** - ATCC® 136-XV™
  - Don’t have time to test your cell lines? Let the experts in mycoplasma detection handle it. We offer a PCR-based testing service that can quickly and reliably detect over 60 species of *Mycoplasma, Acholeplasma, Spiroplasma*, and *Ureaplasma*.

ATCC®

Universal Mycoplasma Detection Kit

ATCC® 30-1012K™

Grow cells	Scrape and suspend cells, or count	Add Lysis Buffer	Perform PCR	Run gel
				
Works with adherent and suspension cells. Use 10^4 to 10^5 cells per assay.	Transfer cell suspension to Sample Lysis Tubes. Pellet cells. Cell pellet can be stored until ready to assay.	Incubate. Heat at 95°. Spin. Transfer cell lysate to new microfuge tube. Cell lysate can be stored until ready to assay.	Perform optimized touchdown PCR using Universal PCR + Primers Mix.	Clear results in less than 3 hours.

- Detects over 60 species of *Mycoplasma, Acholeplasma, Spiroplasma,* and *Ureaplasma*
- All components for the PCR reaction are provided and optimized for amplification

ATCC® sera

Current offerings

ATCC® number	Product name	Volume
30-2020™	Fetal Bovine Serum	500 mL
30-2021™	Fetal Bovine Serum	100 mL
SCRR-30-2020™	Fetal Bovine Serum, ES Qualified	500 mL
30-2030™	Calf Bovine Serum	500 mL
30-2031™	Calf Bovine Serum	100 mL

- Triple filtered with 0.1 µm filters
- Tested for sterility
  - Bacterial and fungi tested compliant with current USP recommendations
  - Virus testing compliant with 9 CFR 113.53
- Endotoxin testing
  - Important to ensure cells are not inadvertently activated/stimulated.
- Growth promotion
  - Each lot is tested to support the growth of several different cell lines using both sequential growth curves and plating efficiencies.
  - ES FBS is tested for its ability to support the growth and maintenance of embryonic stem cells in an undifferentiated state.
- Collected in USDA inspected-abattoirs located in the United States.

ATCC® animal by-product free reagents

Current offerings

- Cryopreservation reagents:
  - Stem Cell Freezing Media
  - Serum Free Freezing Medium
- Dissociation reagents:
  - Stem Cell Dissociation Reagent
  - Non-Enzymatic Cell Dissociation Solution
  - Soybean Trypsin Inhibitor
- Growth reagents:
  - L-Glutamine
  - DMEM,EMEM, DMEM:F12, F12K, IMDM, L-15, RPMI-1640……..

  And newly added: McCoy's 5A, ABP-Free

ATCC® number	Product name
30-2002™	Dulbecco's Modified Eagle's Medium (DMEM)
30-2006™	DMEM:F12 Medium (1:1 Ratio)
30-2003™	Eagle's Minimum Essential Medium (EMEM)
30-2004™	F12K Medium
30-2005™	Iscove's Modified Dulbecco's Medium (IMDM)
30-2008™	Leibovitz's L-15 Medium
30-2001™	RPMI-1640 Medium
30-2011™	McCoy's 5A, ABP-Free
30-2103™	Non-Enzymatic Cell Dissociation Solution
30-2104™	Soybean Trypsin Inhibitor
30-2200™	Dulbecco's Phosphate Buffered Saline (DPBS)*
30-2213™	Hank's Balanced Salt Solution (HBSS)*
PCS-999-001™	Phenol Red
4-X™	Dimethylsulfoxide (DMSO)
30-2600™	Serum-Free Cell Freezing Medium

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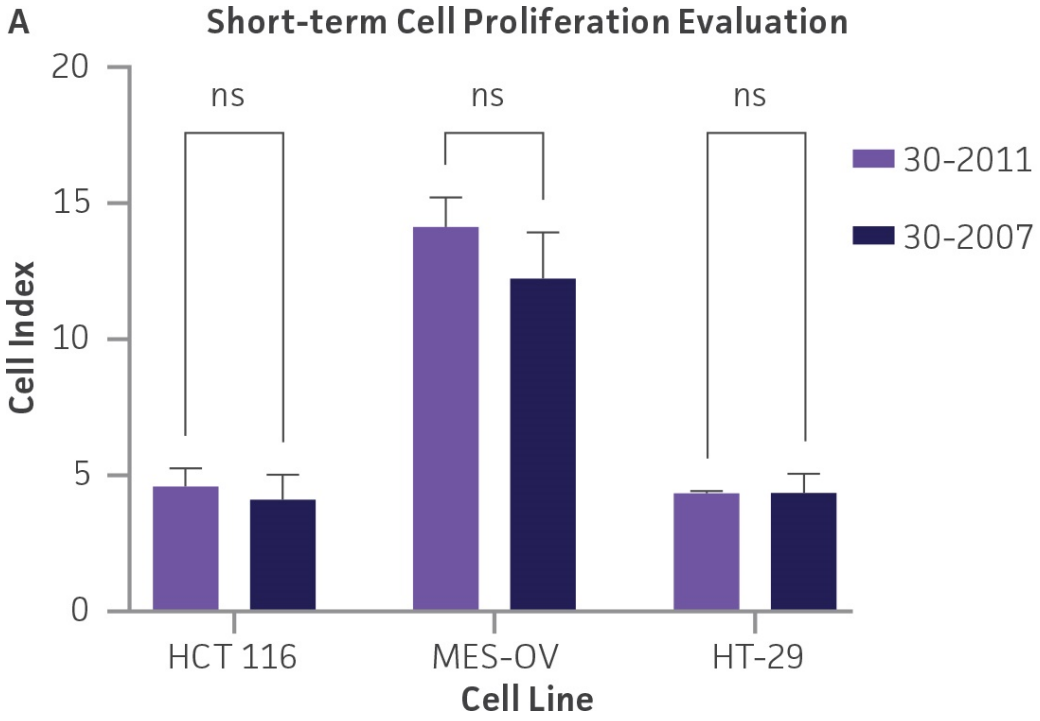
ATCC® animal by-product free reagents – *new!*

New offerings

- ATCC® has developed a modified animal by-product free McCoy's 5A medium formulation: McCoy's 5A Medium, ABP-Free (ATCC® 30-2011™)
- Previous formulation contains Bacto™ peptone.
- Benefits:
  - Animal by-product free, reduces risk to process/product with the removal of Bacto™ peptone.
  - No adaption needed to transition from ATCC® 30-2007™ to ATCC® 30-2011™
  - Supports cell health and proliferation.
  - New cell banks grown in ATCC® 30-2011™ for full end to end solution

ATCC® animal by-product free reagents – *new!*

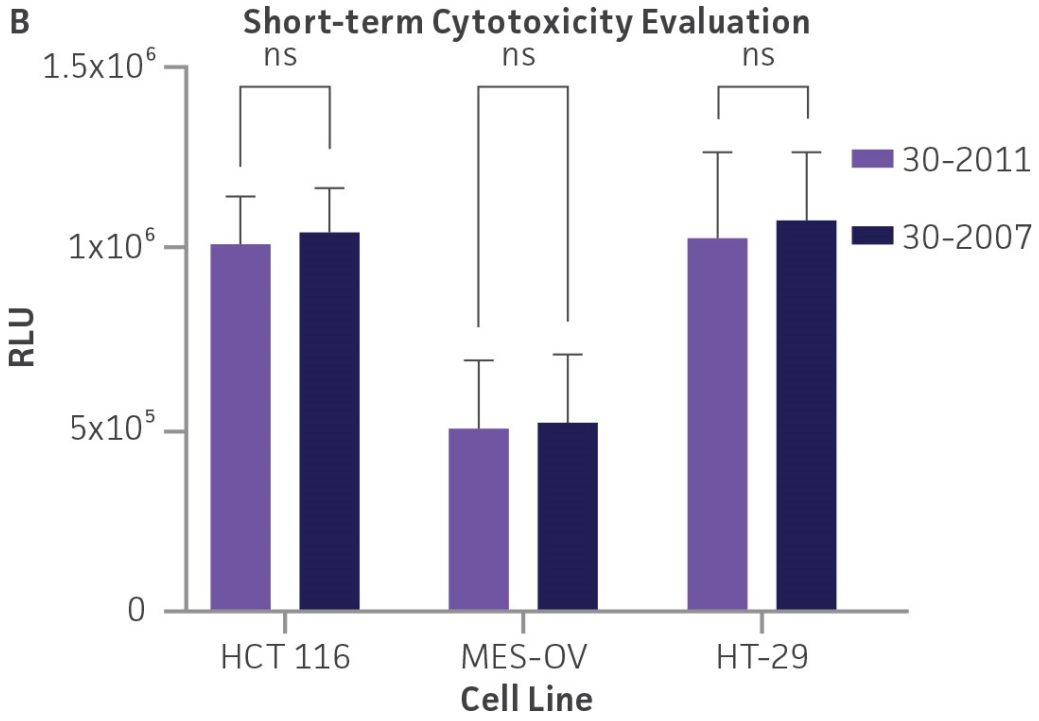
Development of McCoy's 5A, ABP-Free



- 3 cell lines were tested
  - Selected on ability to form uniform monolayers
- Short-term proliferation measured using xCELLigence® RTCA system (Agilent®)
  - Cell index directly correlates to confluence/cell size

ATCC® animal by-product free reagents – *new!*

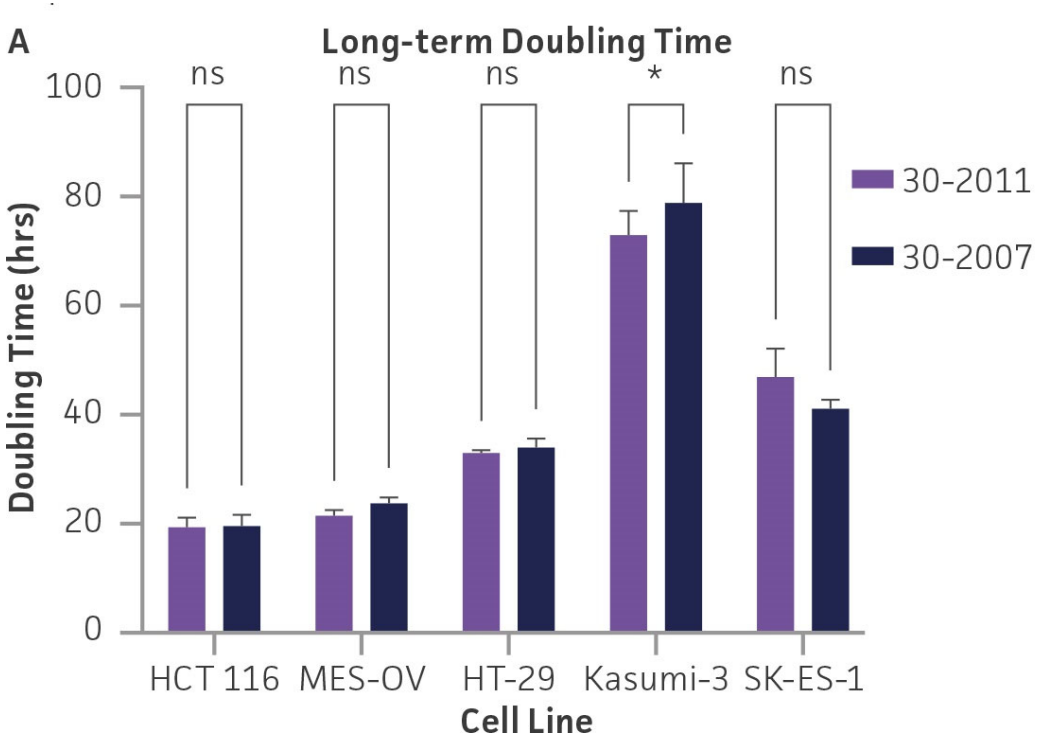
Development of McCoy's 5A, ABP-Free



- 3 cell lines were tested
- CellTiter-Glo® assay
  - Relative luminescence signal corresponds to ATP content
- ATCC® 30-2011™ and ATCC® 30-2007™ performed equivalently

ATCC® animal by-product free reagents – *new!*

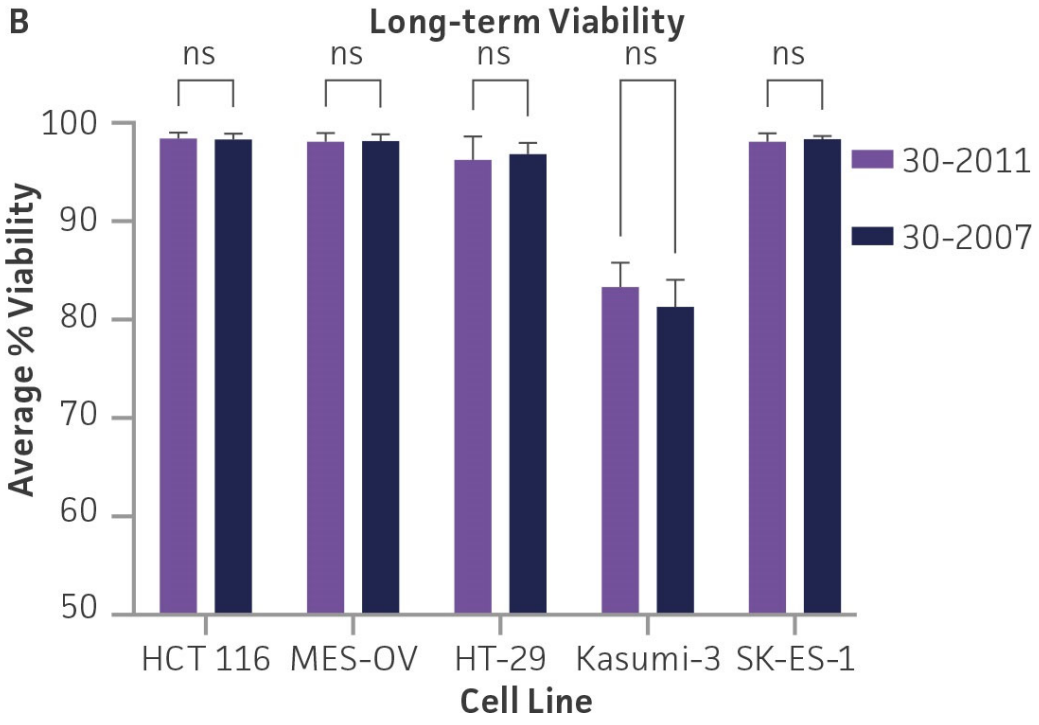
Development of McCoy's 5A, ABP-Free



- 5 cell lines were tested
- Grown for 21-23 days in tissue culture flasks
- Doubling time calculated at each subculture
- ATCC® 30-2011™ and ATCC® 30-2007™ performed equivalently, aside from Kasumi-3 which performed better in ATCC® 30-2011™

ATCC® animal by-product free reagents – *new!*

Development of McCoy's 5A, ABP-Free



- 5 cell lines were tested
- Grown for 21-23 days in tissue culture flasks
- Viability analysis performed at each subculture via automated trypan blue exclusion method
- ATCC® 30-2011™ and ATCC® 30-2007™ performed equivalently for each cell line

ATCC® animal by-product free reagents – *new!*

New offerings

In support of our EU based customers, ATCC® has reformulated the following cell lines with the new McCoy's 5A, ABP to comply with the changing regulatory landscape of the EU.

- MES-SA (ATCC® CRL-1976.NM™)
- ME-180 (ATCC® HTB-33.NM™)
- HEC-1-A (ATCC® HTB-112.NM™)
- MES-SA/MX2 (ATCC® CRL-2274.NM™)
- Malme-3 (ATCC® HTB-102.NM™) – *Coming soon!*

Conclusion

Media Selection is Critical for Cell Biology Workflows

- Animal by-products should be avoided when selecting cell biology reagents, when possible.
- ATCC® offers several animal by-product free solutions and is expanding our offering with the new McCoy's 5A, ABP-Free formulation.
- ATCC® is remanufacturing its McCoy's media cell lines in the xeno-free version to better support the scientific community in navigating the safety and regulatory challenges associated with animal by-product-containing cell lines.
- ATCC® is collecting additional NGS data in support of these new offers to be available to customers beginning in Q3.