acCELLerate

How to Control and to Maintain the Quality of Cell Cultures

ASCCT Webinar

November 30th, 2021

acCELLerate

cell culture models

balancing multiple plates

availability

predictivity

Cell Lines **Stem Cells** **Primary Cells** **Tissues**

Monolayer Cells **Spheroids** **Organoids** **Organ-on-a-Chip**

reproducibility

complexity

ASCCT Webinar

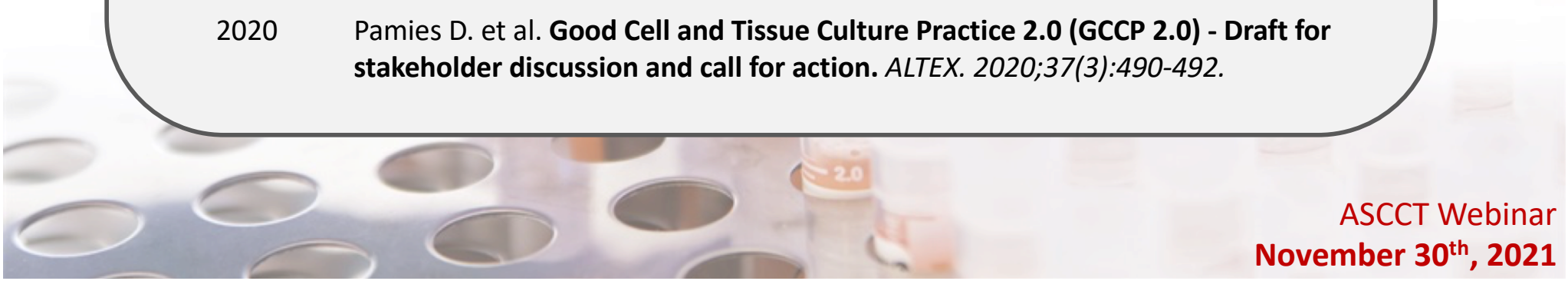
November 30th, 2021

accELLerate

good cell culture practice

GCCP & GIVIMP

2002	Hartung, T. et al. **Good cell culture practice. ECVAM good cell culture practice task force report 1**. *Altern Lab Anim 30, 407-414.*
2005	Coecke, S. et al. **Guidance on good cell culture practice – A report of the second ECVAM task force on good cell culture practice.** *Altern Lab Anim 33, 261-287.*
2017	Pamies D. et al. **Good Cell Culture Practice for stem cells and stem-cell-derived models.** ALTEX. 34(1):95-132.
2018	Pamies D. et al. **Advanced Good Cell Culture Practice for human primary, stem cell-derived and organoid models as well as microphysiological systems.** *ALTEX. 5(3):353-378*
2018	OECD. **Guidance Document on Good In Vitro Method Practices (GIVIMP).** OECD Series on Testing and Assessment, No. 286. OECD Publishing, Paris.
2020	Pamies D. et al. **Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0) - Draft for stakeholder discussion and call for action.** *ALTEX. 2020;37(3):490-492.*



ASCCT Webinar

November 30th, 2021

acCELLerate

cells are alive

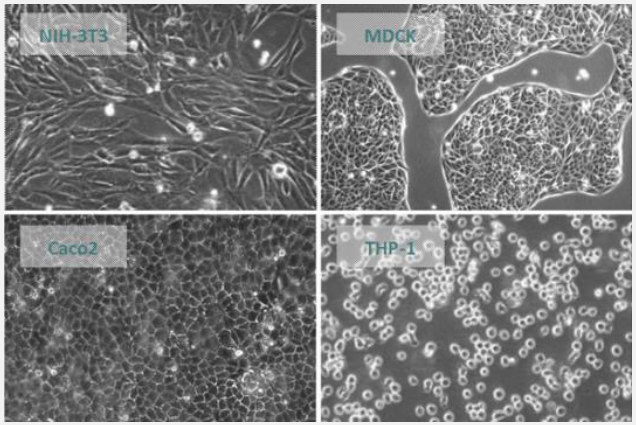
determinants of cell quality

storage

cultivation

medium & serum

identity



culture vessels

sterility

equipment

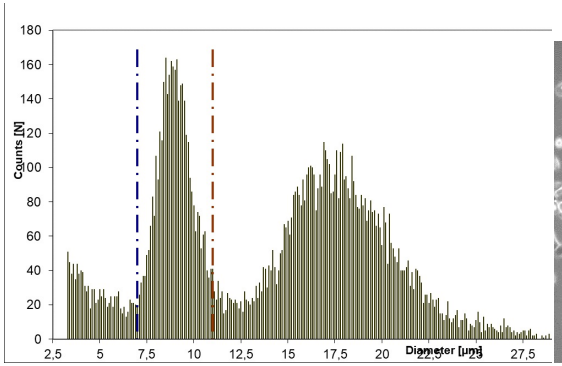
stability

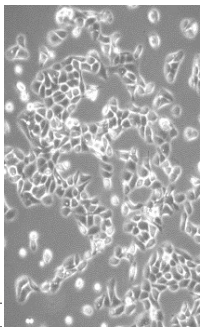
accELLerate

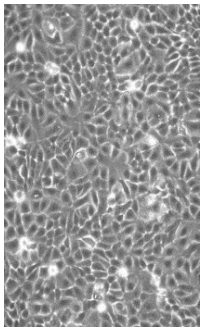
cultivation

you'll get what you deserve

- Viability & Debris
- Aggregation
- Confluence / Density
- Growth Rate
- Morphology







Date / Time	Growth Rate [µ]	Detachment [min.]	Culture Dish [cm²]	Total Area [cm²]	Harvest Density [c/cm²]	Viable Cell Number	Viability [%]	Aggregation	Debris / Cell	Passage	Comment
09.03.21 11:44 AM						5,96E+06	97,8	1,38	0,2	57	Seeding from MCB
11.03.21 7:35 AM	0,60	5	TC75	225	7,96E+04	1,79E+07	97,8	1,23	0,1	58	
13.03.21 11:38 AM	0,44	5	TC175	700	6,69E+04	4,68E+07	97,8	1,22	0,2	59	
16.03.21 08:30	0,44	5	CS6360	3180	5,25E+04	1,67E+08	98,1	1,24	0,1	60	
19.03.21 7:28 AM	0,46	5	CS6360	9540	6,75E+04	6,44E+08	98,1	1,34	0,2		Harvest of WCB

ASCCT Webinar

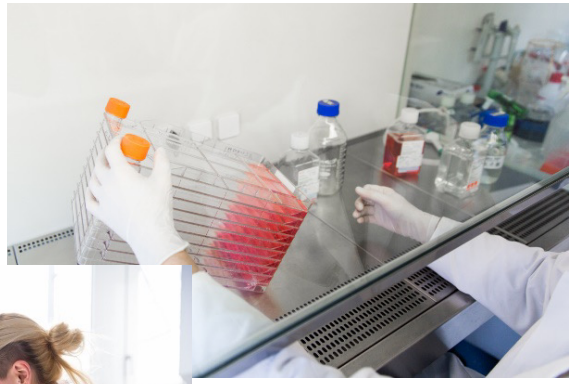
November 30th, 2021

accELLerate

cultivation

you'll get what you deserve

- Follow the SOP
- Train the Operator
- Monitor the Cell Quality
- Document the Process



ASCCT Webinar

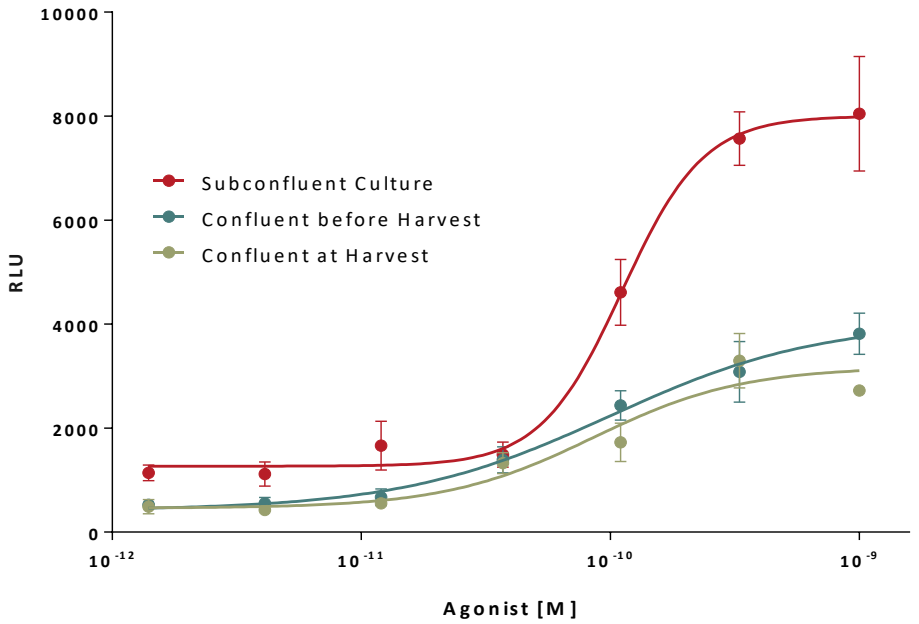
November 30th, 2021

acCELLerate

cultivation

you'll get what you deserve

- Follow the SOP
- Train the Operator
- Monitor the Cell Quality
- Document the Process



Cell Line: **PC3-NHR-Luc**
Target: **Nuclear Hormone Receptor**
Assay: **Luciferase reporter gene assay**

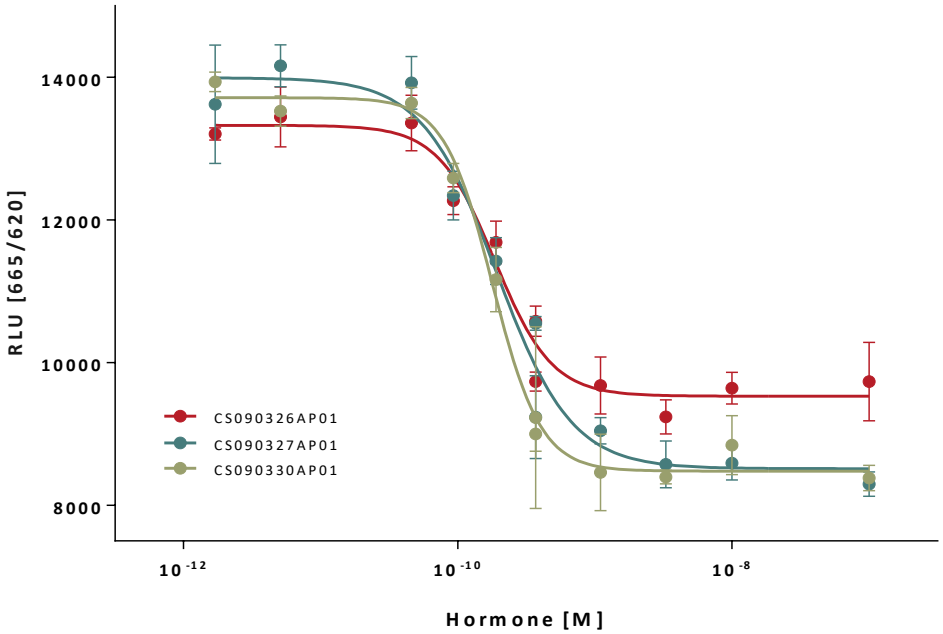
ASCCT Webinar
November 30th, 2021

accELLerate

cultivation

you'll get what you deserve

- Follow the SOP
- Train the Operator
- Monitor the Cell Quality
- Document the Process



Cell Line: **assay ready CHO-GPCR**
Target: **Hormone Receptor**
Assay: **IP-One htrf Assay**

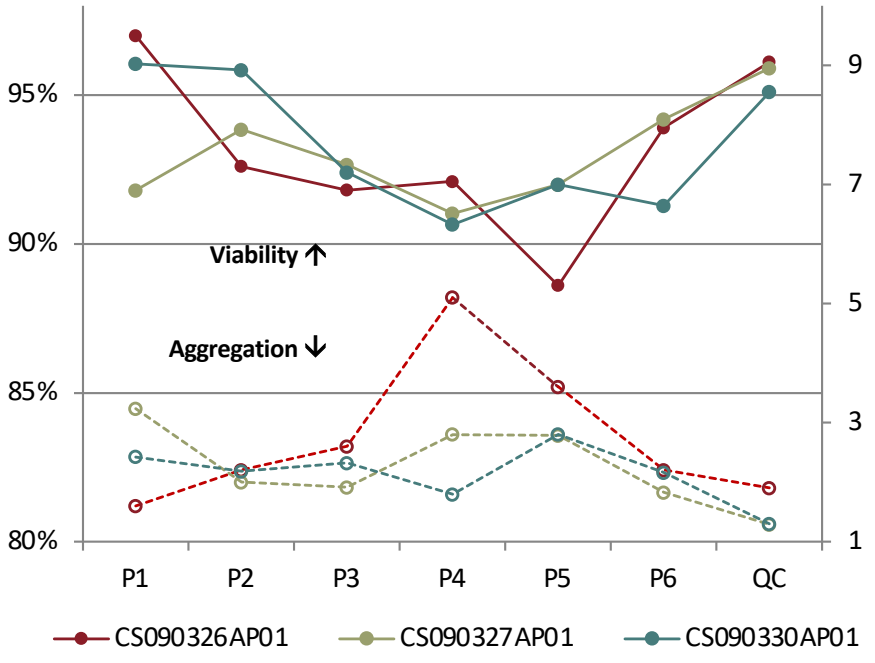
ASCCT Webinar
November 30th, 2021

accELLerate

cultivation

you'll get what you deserve

- Follow the SOP
- Train the Operator
- Monitor the Cell Quality
- Document the Process



ASCCT Webinar

November 30th, 2021

acCELLerate

consumables & equipment

qualify & control

- **Culture Vessels**
- **Media**
- Serum
- Incubators

ASCCT Webinar

November 30th, 2021

accELLerate

consumables & equipment

qualify & control

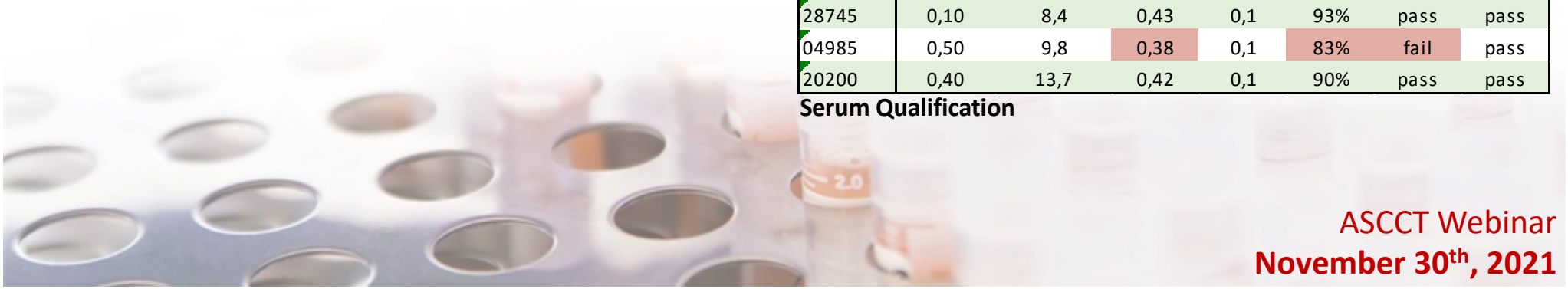
- Culture Vessels
- Media
- **Serum**
- Incubators

Serum

- Stimulates cell proliferation and cell attachment. Buffers toxic substances.
- Level of Endotoxin & Hemoglobin
- Sterility, Virus & Mycoplasma tested
- Replace serum if possible. If you cannot ty to reduce serum levels.

Supplier Lot	Endotoxin [EU/ml]	Hemoglobin [mg/dl]	Jurkat Growth	Jurkat Debris	Raji Viability	Caco2 (TEER)	THP-1 MAT
03275	0,73	25,2	0,42	0,1	89%	fail	pass
38754	5,43	11,1	0,37	0,3	94%	pass	fail
87232	3,10	11,4	0,33	0,3	94%	pass	pass
28745	0,10	8,4	0,43	0,1	93%	pass	pass
04985	0,50	9,8	0,38	0,1	83%	fail	pass
20200	0,40	13,7	0,42	0,1	90%	pass	pass

Serum Qualification



ASCCT Webinar

November 30th, 2021

accELLerate

consumables & equipment

qualify & control

- Culture Vessels
- **Media**
- Serum
- **Incubators**

5 % CO_2 is always set …?

- CO_2 in the atmosphere of the incubator influences the pH of the medium.
- The optimal concentration of CO_2 depends on the buffer capacity of the medium, i.e. the concentration of $NaHCO_3$.

pH at 5% CO_2:

- RPMI 1640 (2.0 g/L) pH 7.43
- DMEM (3.7 g/L) pH 7,69
- Ham's F12 (1,176 g/L) pH 7,19

https://www.cellseeker.org/cellcalc/co2-calculator/

acCELLerate

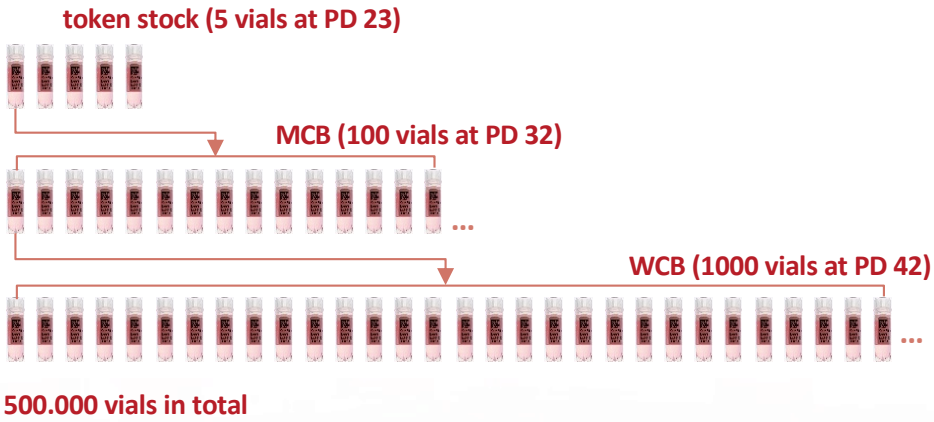
stability

face that final curtain

- **Aging of Cell Cultures**
- Marker Expression
- Pluripotency of stem cells

Aging of Cells

- Finite vs. infinite (immortal) cell lines
- Passage vs. Population Doubling
- Establish a cell banking system



ASCCT Webinar

November 30th, 2021

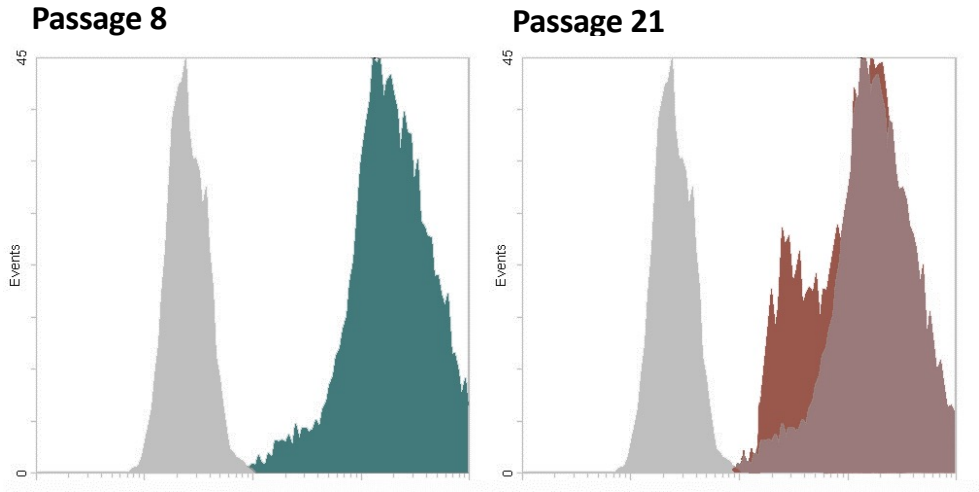
acCELLerate

stability

face that final curtain

- Aging of Cell Cultures
- **Marker Expression**
- Pluripotency of stem cells

Control of Marker Expression



Expression of a recombinant surface marker expressed in HK293 cells at passage 8 (green) and at passage 21 (red). Isotype control (grey)

acCELLerate

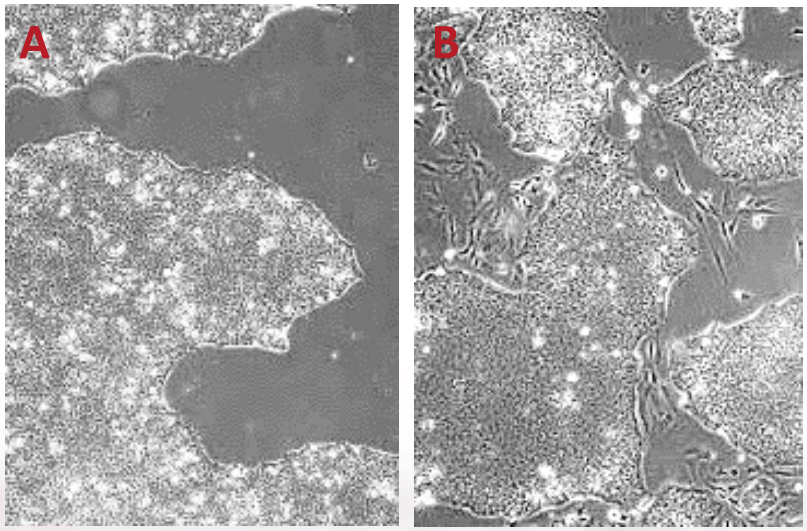
stability

face that final curtain

- Aging of cell cultures
- Marker Expression
- **Pluripotency of stem cells**

Scoring of iPSC colonies

- A: compacted iPSC colonies with defined edges; uniform morphology.
- B: iPSC colonies with some differentiation around the edges, cells more loosely packed.



Source: EBiPC

accELLerate

sterility

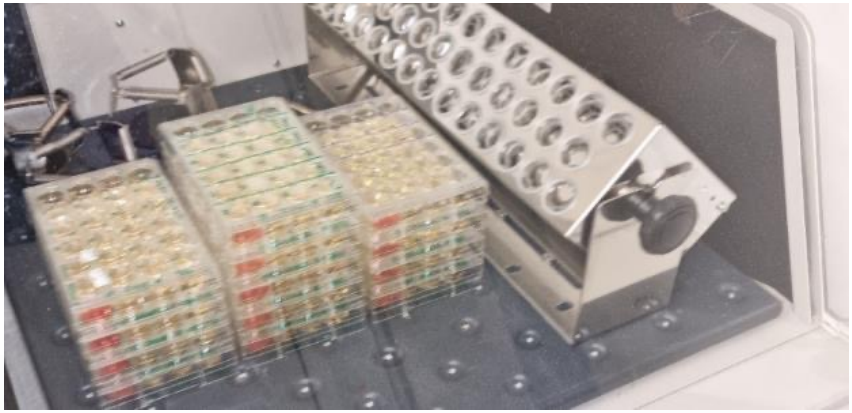
better safe than sorry

- **Bacteria, fungi & yeast**
- Mycoplasma
- Viruses
- How to maintain sterility

Steri Broth Inoculation (7-10 days)

TSB - Tryptic Soy Broth
facultative aerob bacteria

THIO Thioglycollate Broth
facultative anaerob bacteria & yeast



ASCCT Webinar
November 30th, 2021

acCELLerate

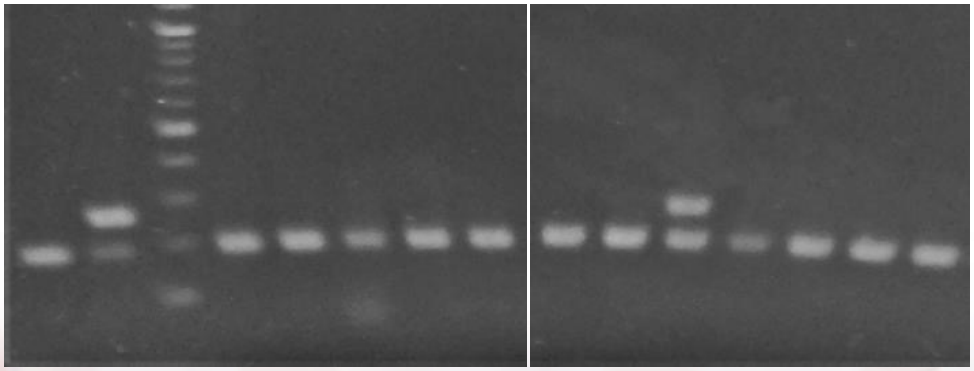
sterility

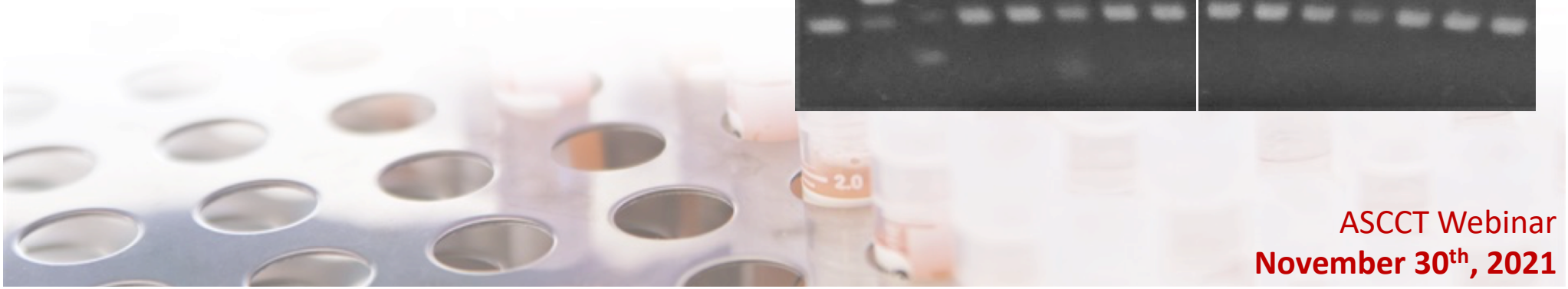
better safe than sorry

- Bacteria, fungi & yeast
- **Mycoplasma**
- Viruses
- How to maintain sterility

Mycoplasma Detection by PCR

- Amplification of 16S rRNA coding region of the mycoplasma genome.
- limit of detection: 20 copies
- detection of M. orale, M. hyorhinis, M. arginini, M. fermentans, M. salivarium, M. hominis, … +85





ASCCT Webinar

November 30th, 2021

acCELLerate

sterility

better safe than sorry

- Bacteria, fungi & yeast
- Mycoplasma
- **Viruses**
- How to maintain sterility

Virus Contamination

- Safety concern: Primary material my contain human pathogenic viruses.
- Adventitious human, bovine, porcine, rodent, and insect viruses
- Viruses from the molecular tools box. Beware of cross contamination.

ASCCT Webinar

November 30th, 2021

accELLerate

sterility

better safe than sorry

- Bacteria, fungi & yeast
- Mycoplasma
- Viruses
- **How to maintain sterility**

Hygiene Measures

- Wear lab coats, gloves and clean shoes.
- Disinfect your hands and instruments. Don’t touch your face.
- Do not use prophylactic antibiotics.
- Ban new or suspicious cultures into a quarantine incubator.
- Discard contaminated cultures immediately.
- Apply a regular hygiene monitoring in the lab.

ASCCT Webinar

November 30th, 2021

accELLerate

identity

what the heck is the HEK

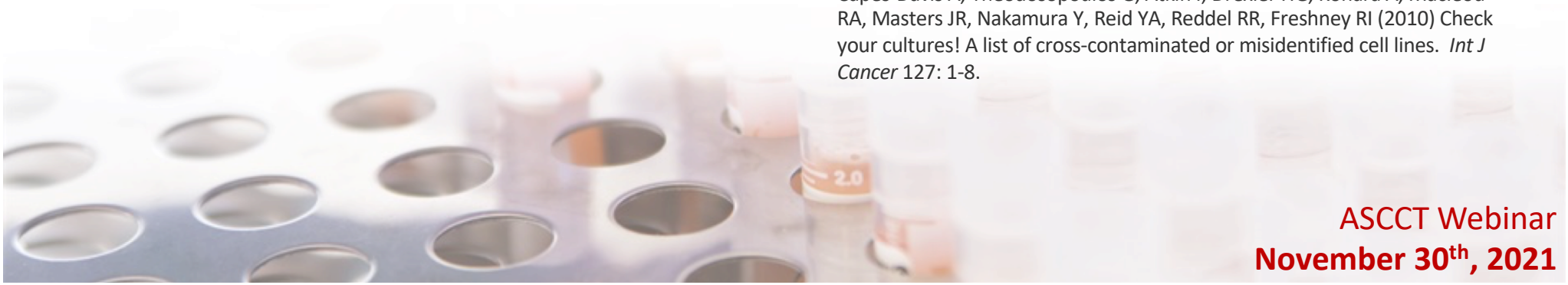
- **Misidentified Cell Lines**
- STR analysis
- Species specific PCR
- How to avoid cross contamination

ICLAC Register of Misidentified Cell Lines

https://iclac.org/databases/cross-contaminations/

- 531 cell lines are misidentified with no known authentic stock. 45 could be retrieved.
- 67 cell lines come from a different species (interspecies contamination)
- 73 cell lines do not correspond to the original donor, but the contaminant is unknown.
- 144 different contaminants are listed. 140 of these are HeLa

Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, Macleod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI (2010) Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer* 127: 1-8.

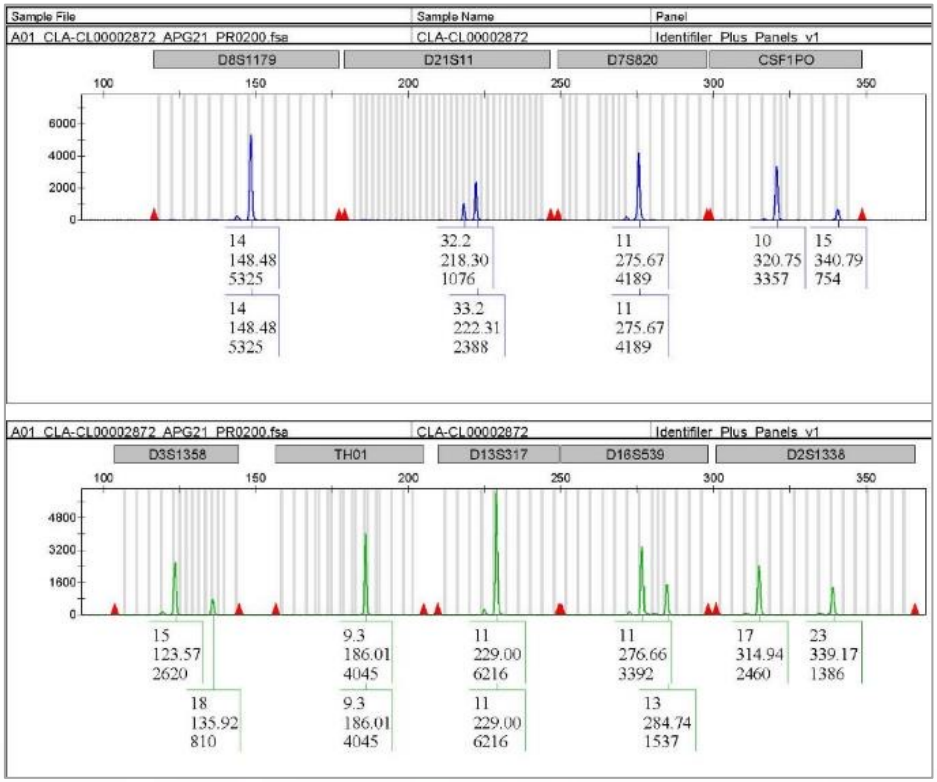


acCELLerate

identity

what the heck is the HEK

- Misidentified Cell Lines
- **STR analysis**
- Species specific PCR
- How to avoid cross contamination



ASCCT Webinar

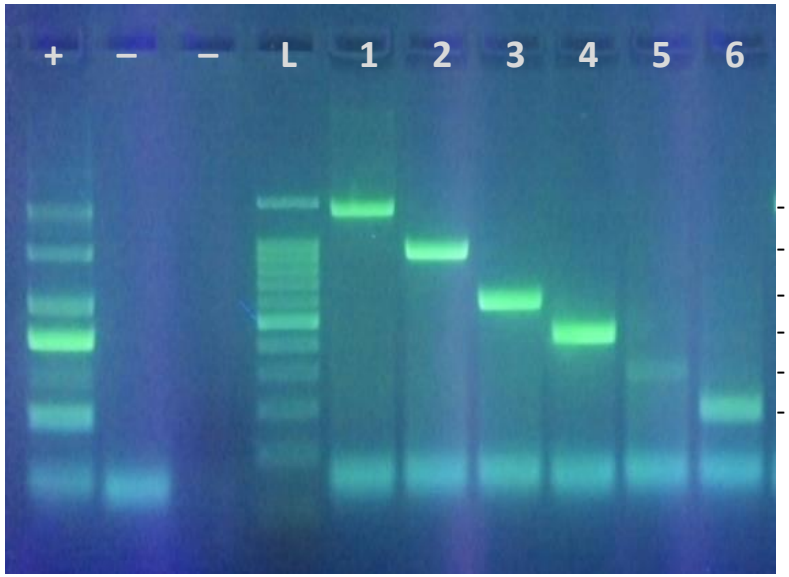
November 30th, 2021

accELLerate

identity

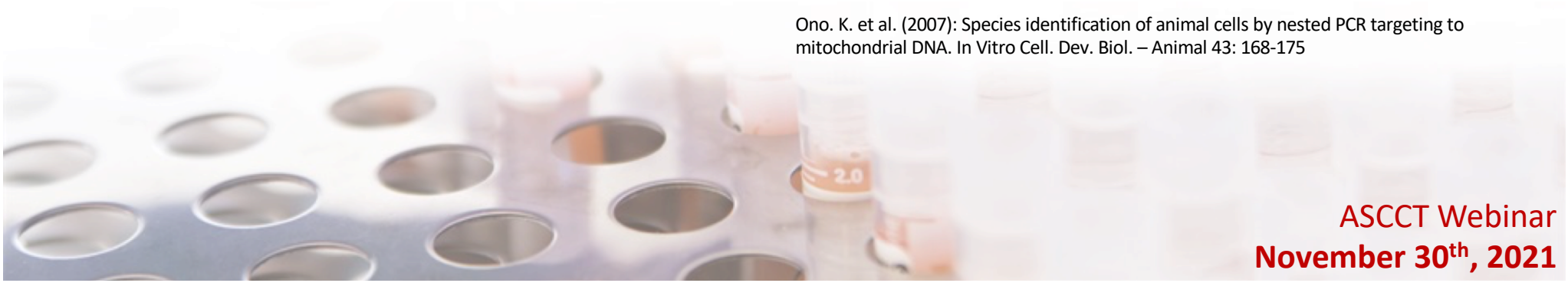
what the heck is the HEK

- Misidentified Cell Lines
- STR analysis
- **Species specific PCR**
- How to avoid cross contamination



1: MDCK (Madin-Darby Canine Kidney)
2: L-929 (Mouse Fibroblasts)
3: CHO-1 (Chinees Hamster Ovary)
4: HEK293 (Human Embryonic Kidney)
5: Vero (African Green Monkey Kidney)
6: H4IIE (Rat Hepatoma Cells)

Ono. K. et al. (2007): Species identification of animal cells by nested PCR targeting to mitochondrial DNA. In Vitro Cell. Dev. Biol. – Animal 43: 168-175



ASCCT Webinar
November 30th, 2021

accELLerate

identity

what the heck is the HEK

- Misidentified Cell Lines
- STR analysis
- Species specific PCR
- **How to avoid cross contamination**

Avoid Cross Contamination

- Apply hygiene measures.
- Don’t handle multiple cell lines at the same time.
- Separate cell lines from each other. Use filter caps.
- Know where your cells are coming from.
- Define the initial status of new cell lines.

Cell Depository | Self Generated | Befriended Laboratory

ASCCT Webinar

November 30th, 2021

acCELLerate

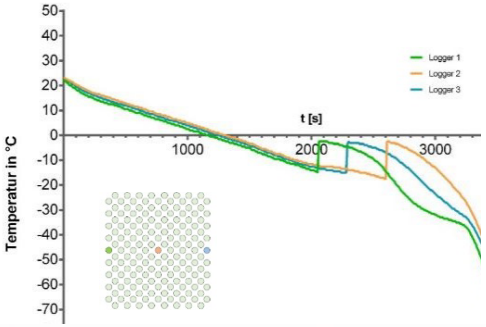
cryopreservation

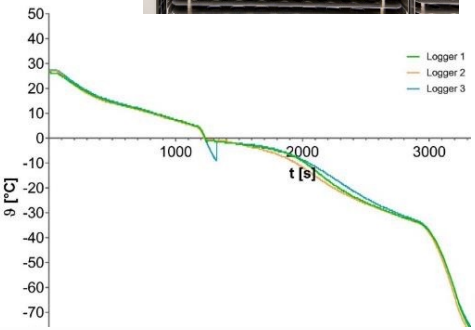
survival of the fittest

- **Freezing**
- Assay Ready Cells
- Storage

Controlled Rate Freezing

Slow cooling and the presence of cryoprotectants (DMSO) prevents the formation of crystals and water becomes an amorphous (non-crystalline) glass.





ASCCT Webinar

November 30th, 2021

accELLerate

cryopreservation

survival of the fittest

- Freezing
- **Assay Ready Cells**
- Storage

Assay Ready Cells

- Cryopreservation should be more than just freezing cells for later recovery; it should preserve the full functionality of cells.
- Optimized freezing media
- Improved cryopreservation protocols
- Turn cells into reagents

ASCCT Webinar

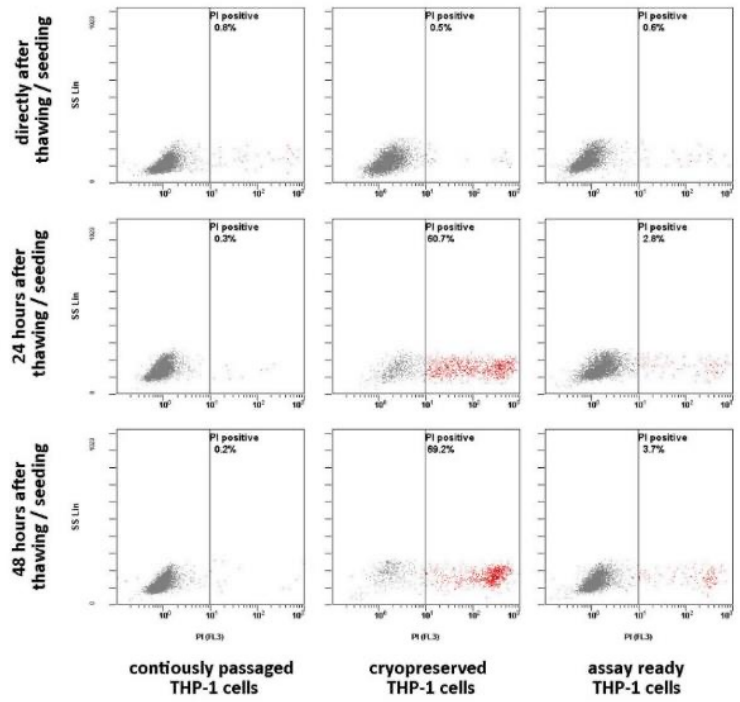
November 30th, 2021

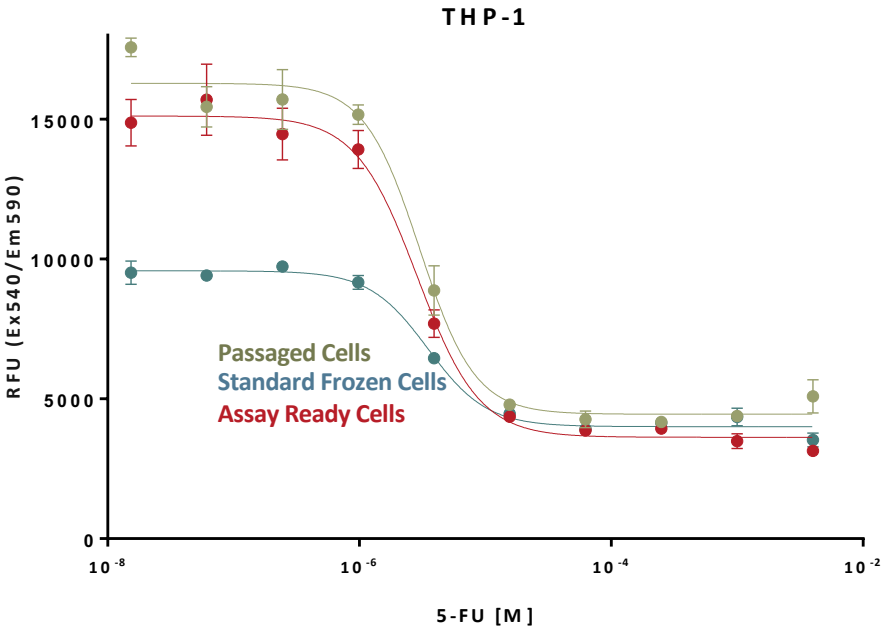
accELLerate

assay ready cells

turning cells into reagents

Recovery of THP-1 Cells



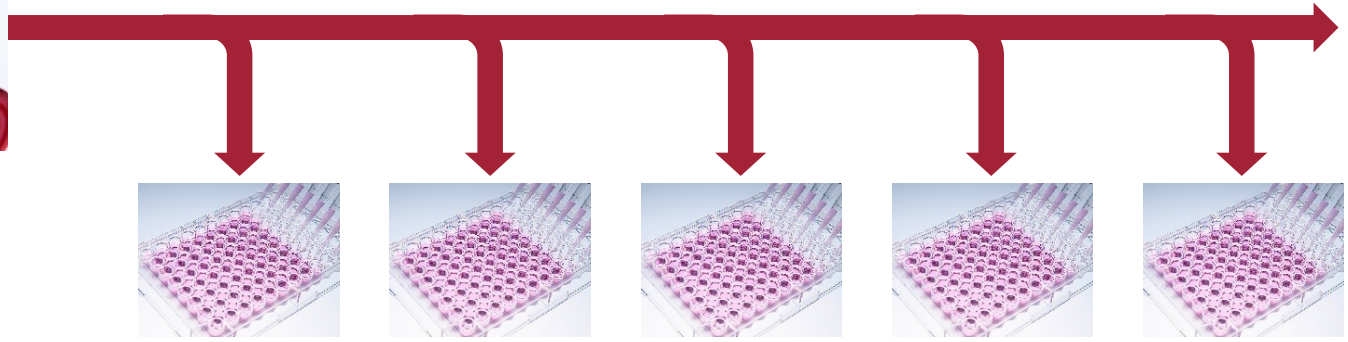


acCELLerate

assay ready cells

turning cells into reagents

Classic Way of Cell Supply



- variability through different handling and lots
- passage drift of cells
- risk of contamination

ASCCT Webinar

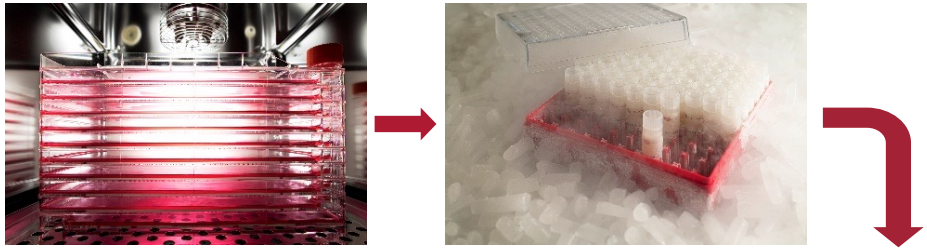
November 30th, 2021

accELLerate

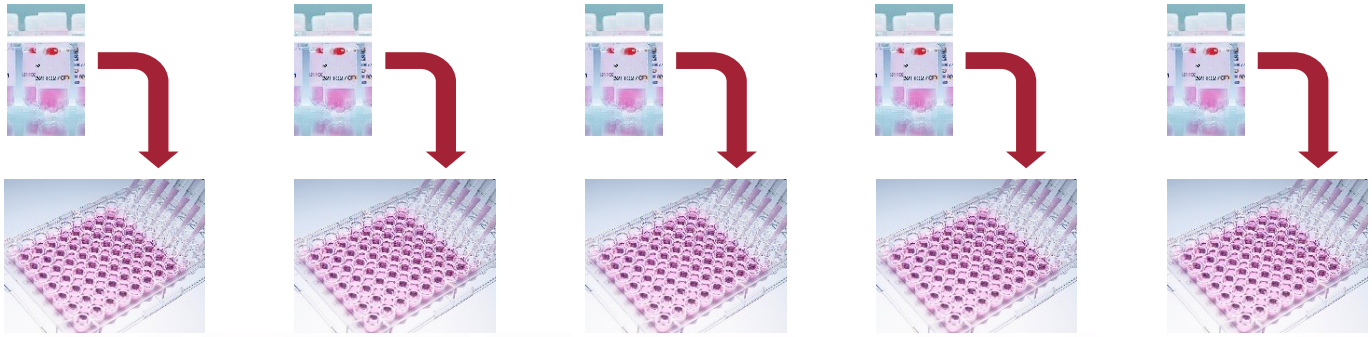
assay ready cells

turning cells into reagents

Smart Way of Cell Supply



prequalified aliquots of assay ready cells



thawing of assay ready cells and instant dispensing into assay plates at the day of use

ASCCT Webinar

November 30th, 2021

acCELLerate

assay ready cells

turning cells into reagents

- Ready to use like a reagent. No cultivation required.
- Harmonizes the impact of cell cultivation, media, and cell age.
- Homogeneous prequalified cell banks increase assay precision.
- Instantly available at any time and at consistent quality.
- Convenient to use even from inexperienced operators.

ASCCT Webinar

November 30th, 2021

acCELLerate

cryopreservation

survival of the fittest

- Freezing
- Assay Ready Cells
- **Storage**

Lost in the Ice

- **- 80°C Deep Freezers** for very short-term storage only.
- **-150°C Ultra Low Freezers**. Beware of Power Failure. liN2 back-up required.
- **Liquid Nitrogen**. Best for long-term storage. Avoid temperature fluctuation above the glass transition point at approx. 137 °C.

ASCCT Webinar

November 30th, 2021

acCELLerate

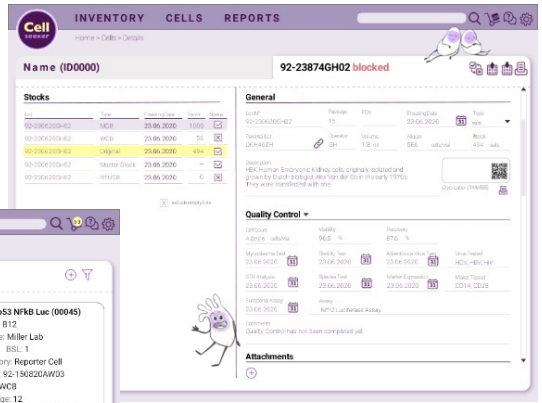
cryopreservation

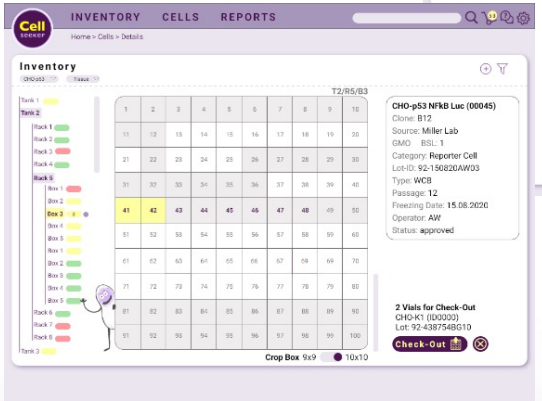
survival of the fittest

- Freezing
- Assay Ready Cells
- **Storage**

Cellseeker Inventory

- Free Inventory Software
- Organizes cells and stocks
- Web based cloud application





www.cellseeker.org
inventory.cellseeker.org/demo

ASCCT Webinar
November 30th, 2021

accELLerate

conclusion

the rule of 5D

Develop acceptance criteria for your cell cultures

Define the limits of acceptance

Detect changes by close observation

Document all cell parameters during cultivation

Discard cells that miss the acceptance criteria