Adenovirus upstream and downstream processing

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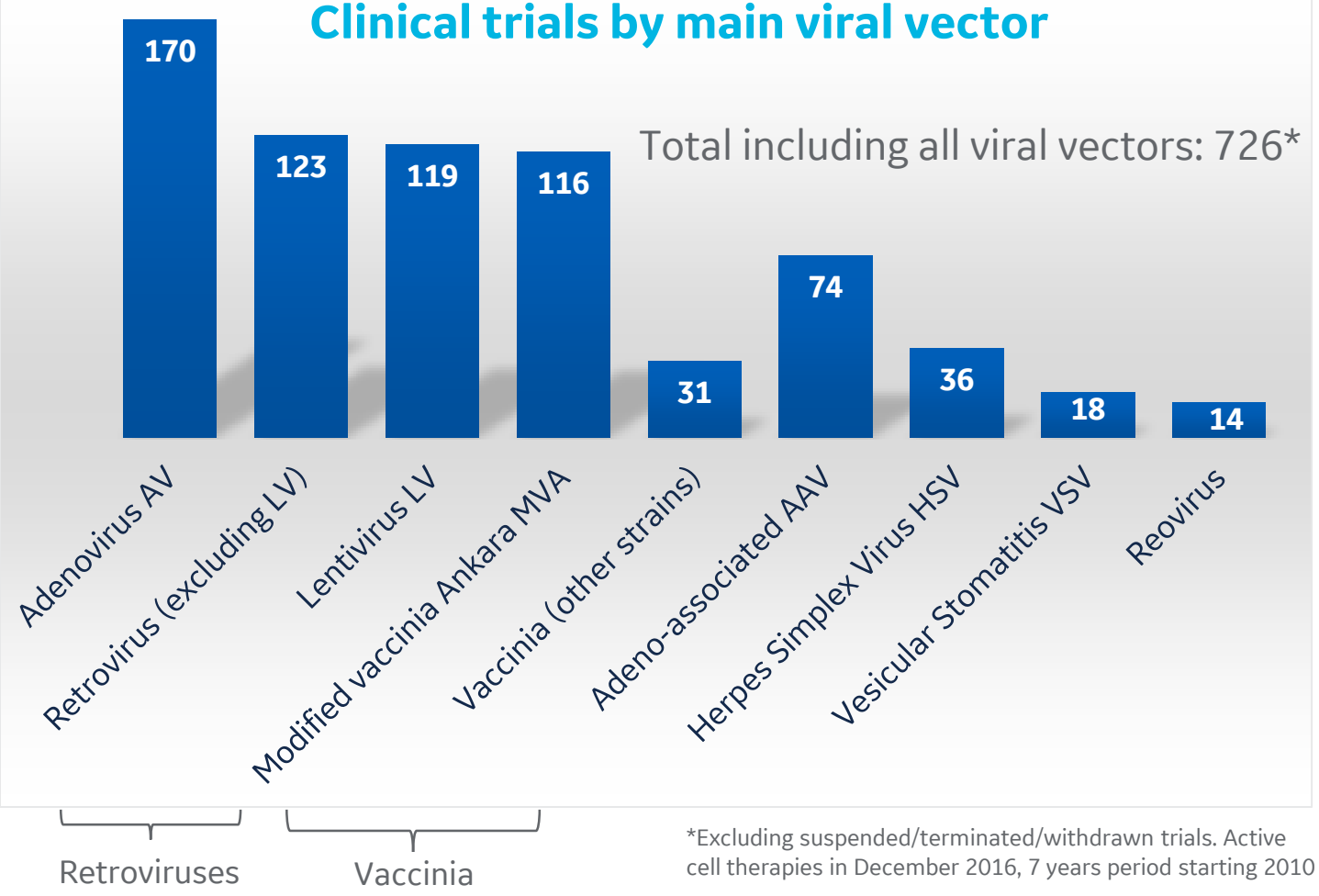
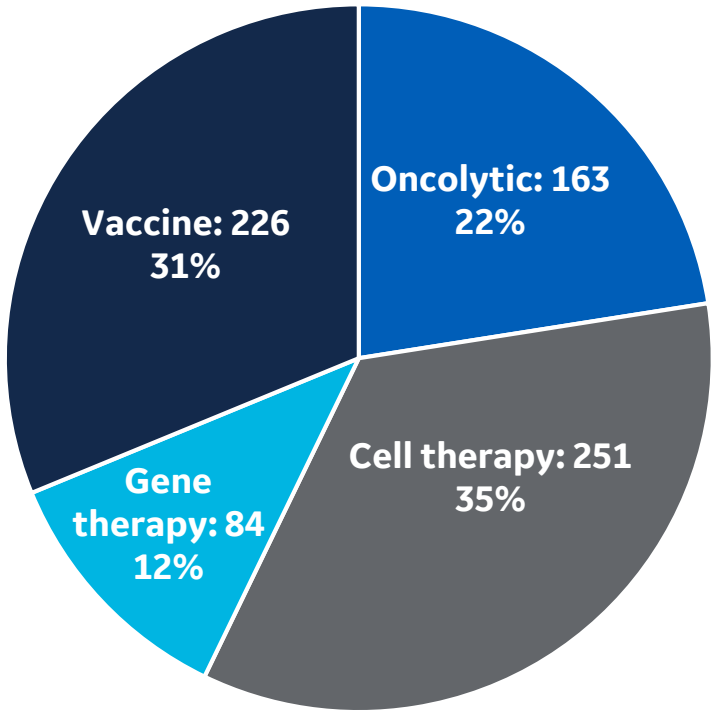
Introduction – Viral Vectors

Number of 2016 active trials—clinical phases globally

Clinical trials by main viral vector



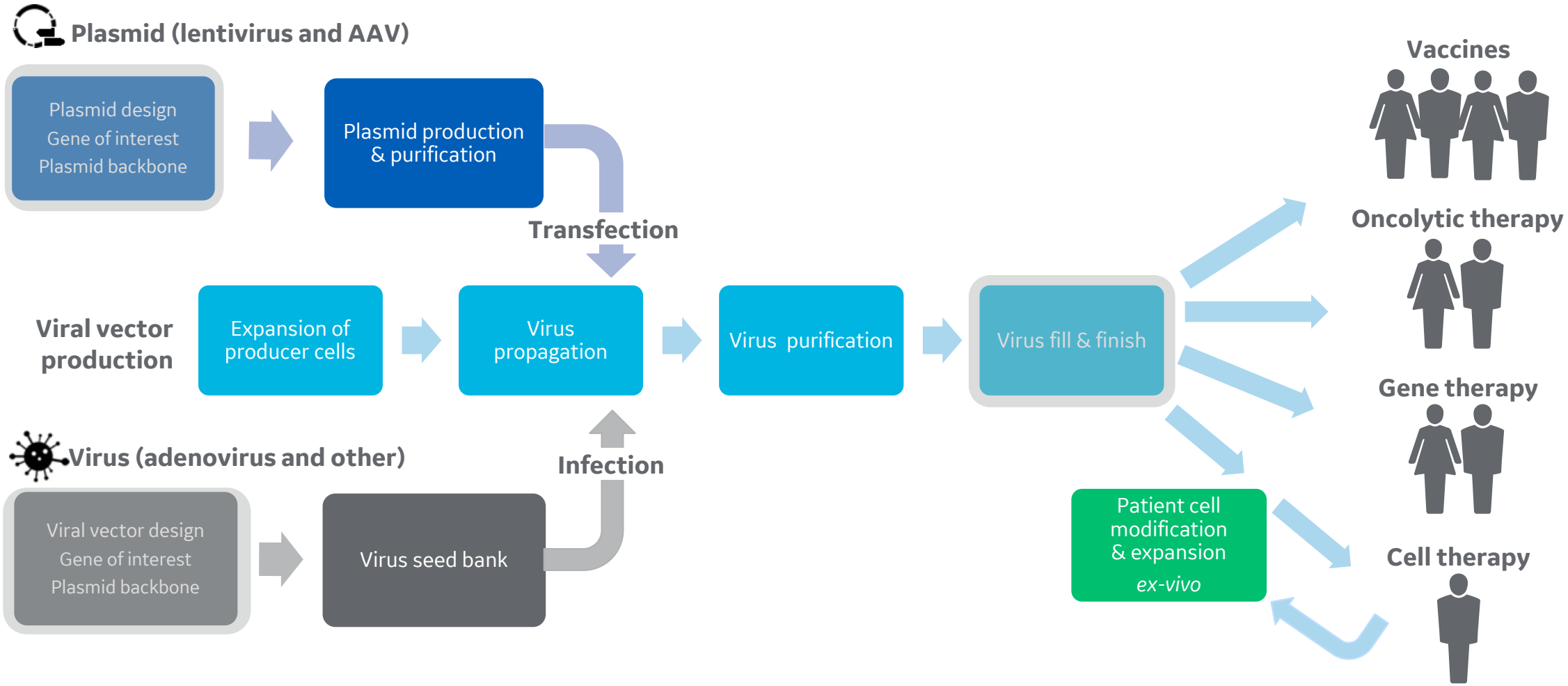
Clinical trials by application



*Excluding suspended/terminated/withdrawn trials. Active cell therapies in December 2016, 7 years period starting 2010

Reference: GE Healthcare internal market analysis

Viral vector production and clinical use



Adenovirus process

Upstream

Xcellerex™ XDR-10

Scaleable in XDR up to 2000 L

ReadyToProcess WAVE™ 25

Downstream

- **Cell lysis DNA fragmentation**
- **Clarification**
- **Conc. and buffer exchange**
- **Capture**
- **Polishing**
- **Conc. and buffer exchange**
- **Sterile filtration**

Analysis

Virus infectivity

% infected cells: flow cytometry

Virus infectious titer

$TCID_{50}$

Automated fluoresence microscopy

IN Cell

Total virus titer

qPCR

Biacore™ system

HPLC

Host cell

DNA: qPCR

Protein: ELISA

Characterization

SDS-PAGE, Western blotting, TEM, Nanosight™, HPLC

WCB = working cell bank

$TCID_{50}$ = 50% tissue culture infective dose

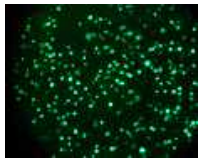
Upstream cell culture and virus propagation

Small scale productivity optimization strategy

Recombinant adenovirus serotype 5—GFP used as model virus propagated in HEK293 suspension cells

Screening of cell culture media

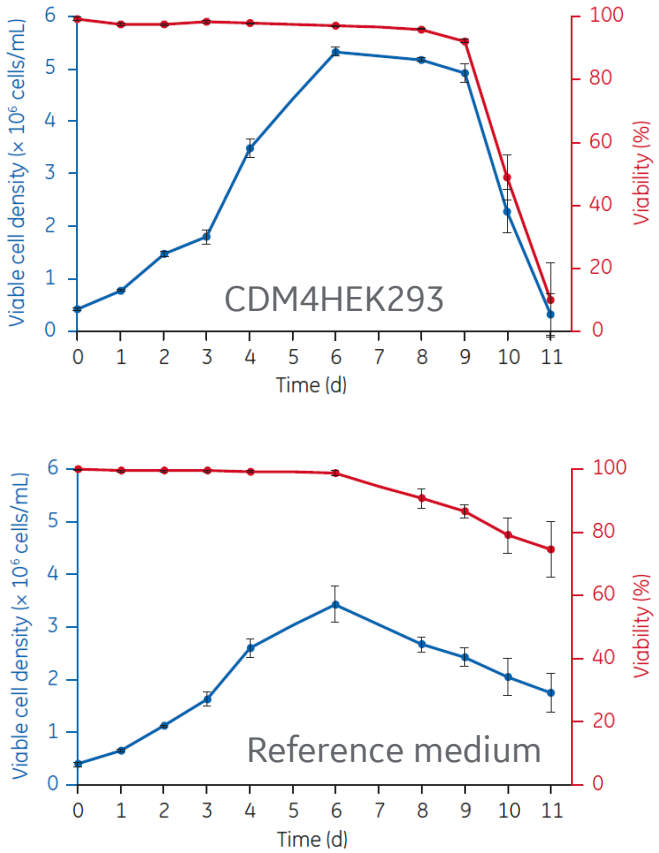
Optimization of MOI, TOI and TOH

Cell culture	Virus infection	Virus propagation	Virus productivity analysis
0 hr	~ 72 hr	~ 120 hr	
 Shake flasks (30–100 mL)	 GFP expression as % infected cells	Cell lysis/ virus release	**Infectious virus titer ivp/mL**
Cell culture medium evaluation **Cell density optimization 0.5–2 × 10^6 cells/mL at TOI**	**Optimization of MOI 0.01–10** **Medium exchange evaluation**	**Optimization TOH 36–72 h**	

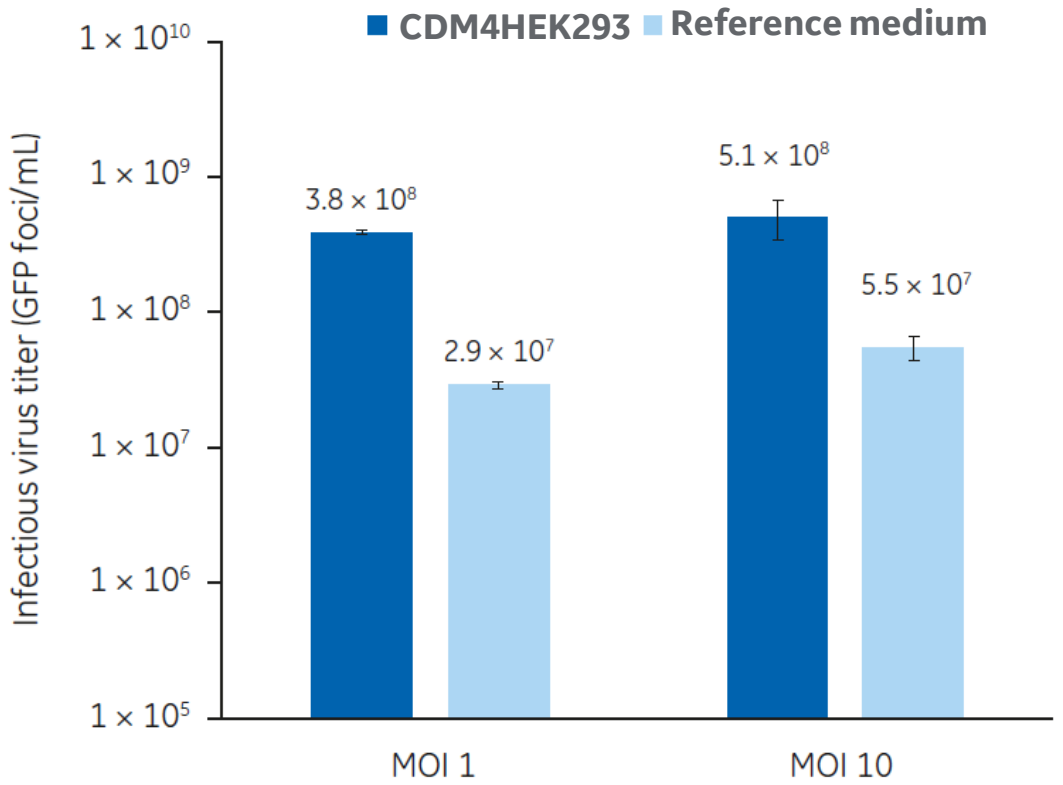
GFP = Green Fluorescent Protein
MOI = multiplicity of infection
TOI = time of infection
TOH = time of harvest
ivp = Infectious virus particle

HyClone™ CDM4 HEK293 cell culture medium was selected

Improved HEK293 cell growth



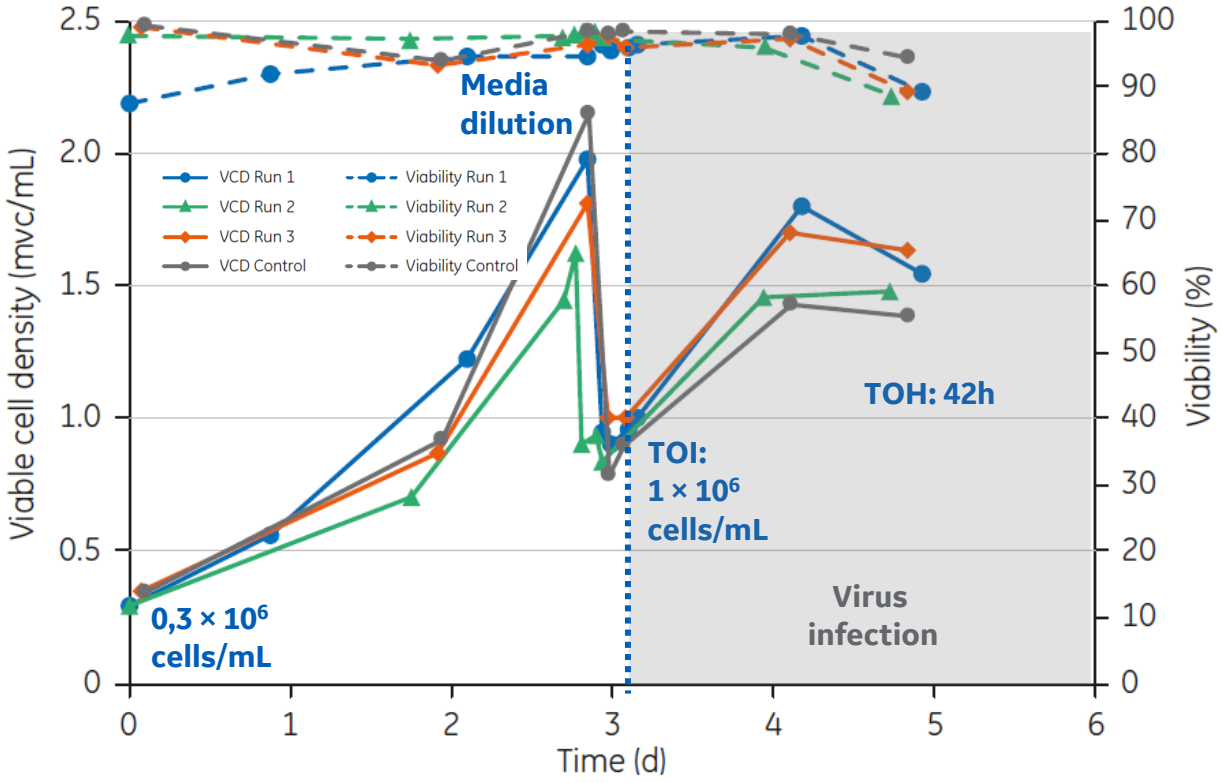
Higher infectious virus titer



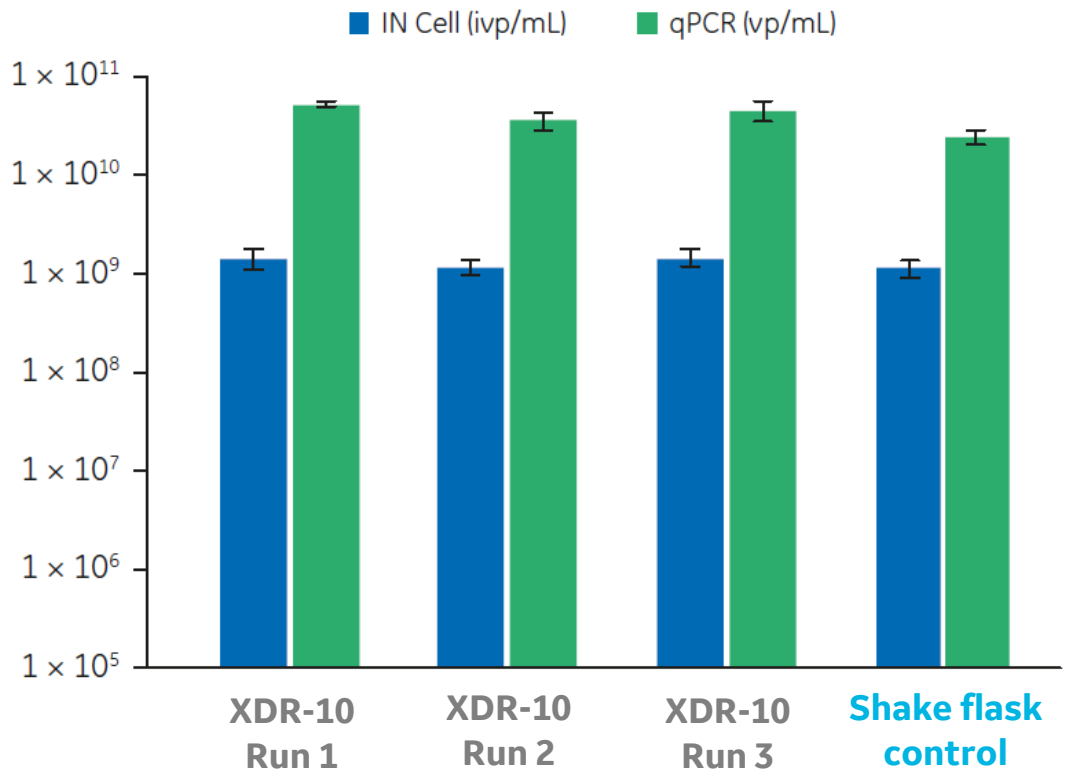
GFP = Green Fluorescent Protein
MOI = multiplicity of infection

Reproducible adenovirus production in Xcellerex™ XDR-10 bioreactor

Cell growth and viability



Adenovirus productivity in XDR-10



ivp = infectious virus particles
TOI = time of infection
TOH = time of harvest

Downstream purification

Evaluation and optimization of each step in small scale

Downstream

- **Cell lysis**
  DNA fragmentation
- **Clarification**
- **Conc. and buffer exchange**
- **Capture**
- **Polishing**
- **Conc. and buffer exchange**
- **Sterile filtration**

Capture: Optimization of elution conditions

Downstream

- Cell lysis DNA fragmentation
- Clarification
- Conc. and buffer exchange
- **Capture**
- Polishing
- Conc. and buffer exchange
- Sterile filtration

Step or gradient elution

Capto™ Q ImpRes with gradient elution

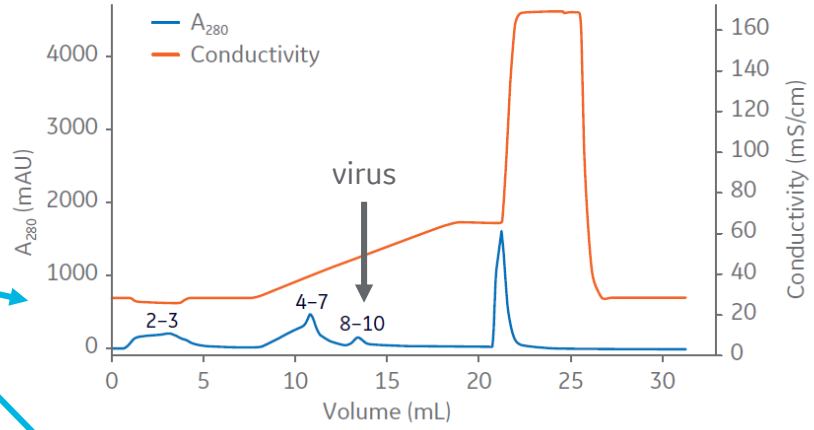
20 mM Tris pH 8, 300–700 mM NaCl

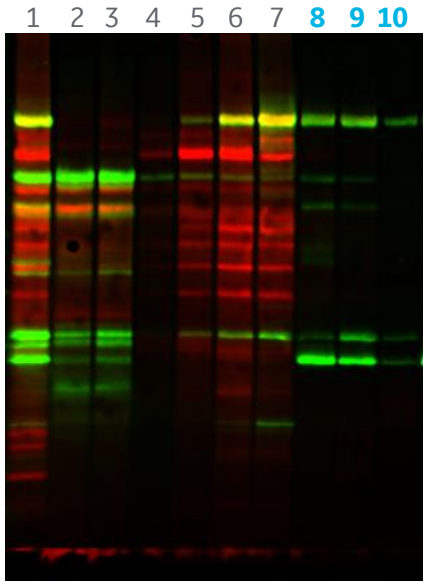
- Highly efficient HCP removal
- Gradient elution improved DNA reduction

ReadyToProcess™ Adsorber Q with step elution

20 mM Tris pH 8, 720 mM NaCl

- Gradient elution not an option, no resolution between virus and impurities





1: start material
2: flowthrough
3: wash
4 to 7: first elution peak (HCP)
8 to 10: elution peak (virus)

Red = HCP
Green = viral proteins

HCP = host cell protein

Polishing: Comparing size exclusion and Capto™ Core 700

Downstream

- Cell lysis DNA fragmentation
- Clarification
- Conc. and buffer exchange
- Capture
- **Polishing**
- Conc. and buffer exchange
- Sterile filtration

Evaluation of capture and polishing combinations, 1 mL HiTrap™ columns

Capture	Polishing	Load	Recovery of total virus particles (%)*	Total protein (µg/dose)	Total DNA (ng/dose)	hcDNA (ng/dose)
Capto Q ImpRes	Sepharose™ 4 Fast Flow	0.1 CV	39/57	< LOD	< LOD	< LOD
	Capto Core 700	26 CV	65/100	< LOD	< LOD	< LOD

** Two numbers indicates that the same sample was analyzed twice.*

Outcome:

- Similar impurity removal performance
- Capto core enables higher sample load volume capacity (up to 30 CV)
- DNA removal after ReadyToProcess™ Adsorber Q capture (step elution) was less efficient for both SEC and Capto Core (data not shown)

CV = column volumes
SEC = size exclusion chromatography

Results for process variants: Analysis of final bulk

Process variant	Recovery vp %	Recovery ivp %	HCP ng/mL	Total protein µg/dose	gDNA ng/dose
Reference process, Run 1	31/38*	36	17	11/13	< LOD
Reference process, Run 2	35/64	53	27	38/20	3
Reference process average	**42**	**45**	**22**	**20**	**< LOD -3**
Novel process, Run 1	46/68	39	< LOD	13/11	< LOD
Novel process, Run 2	17	40	< LOD	10	< LOD
Novel process, Run 3 (10 L)	38/25	50	< LOD	4/10	< LOD
Novel resin process average	**39**	**43**	**< LOD**	**10**	**< LOD**
Membrane process, Run 1	30/44	63	169	30/16	< LOD
Membrane process, Run 2	41/50	28	155	20/3	< LOD
Membrane process average	**41**	**46**	**162**	**17**	**< LOD**

* Two numbers indicates that the same sample was analyzed twice

Purity targets

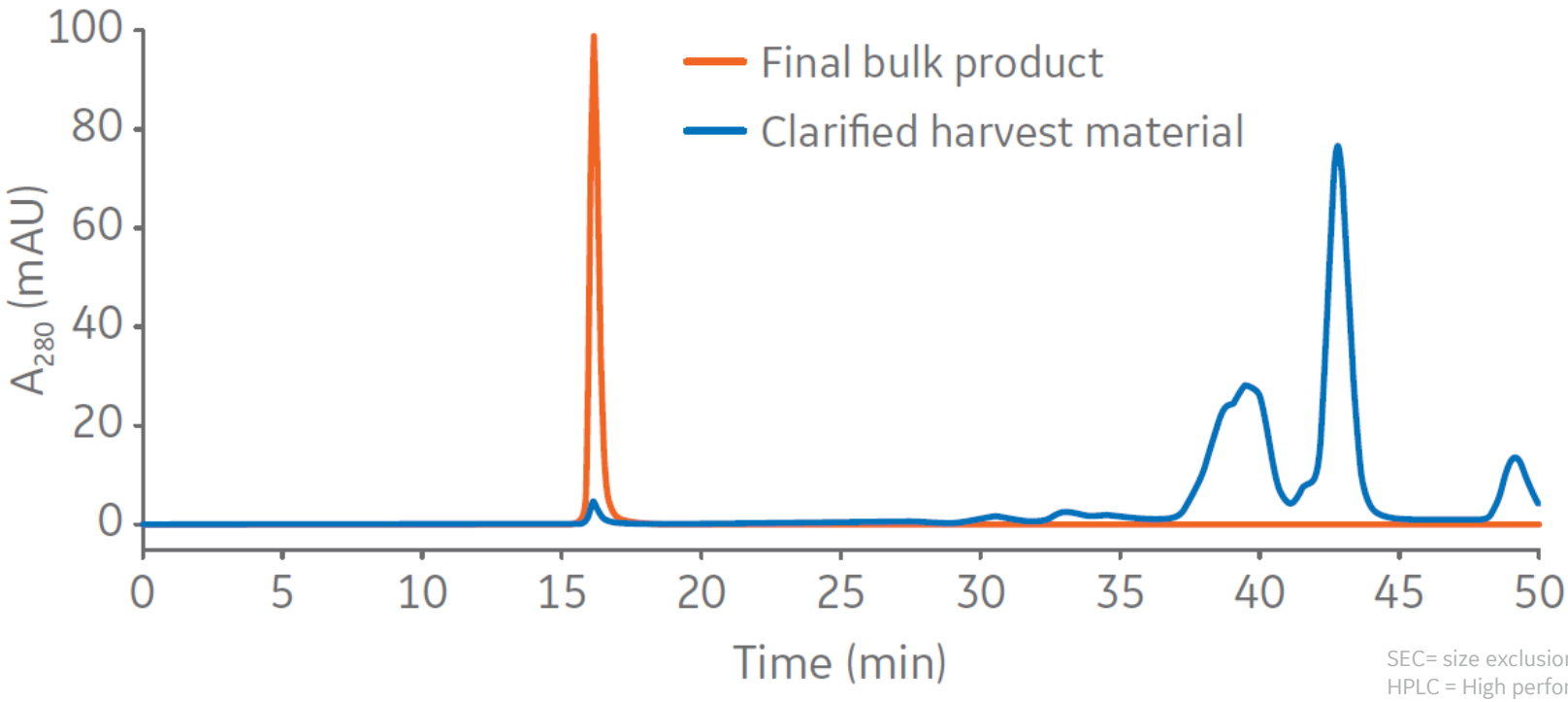
- Dose size assumption: 10^{11} virus particles
- Host cell proteins: < 20 µg/dose
- Host cell genomic DNA: < 10 ng/dose
- Total virus particles/infectious virus particles ratio < 30

< LOD = below limit of detection
vp = Total virus particles (qPCR)
ivp= Infectious virus particles (IN Cell)
HCP = host cell proteins

Characterization

Efficient adenovirus purification and impurity reduction

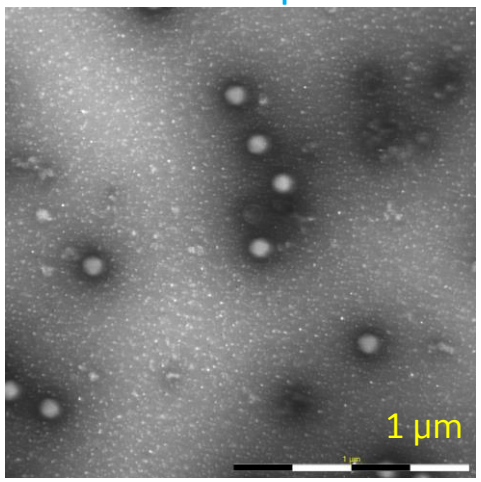
SEC-HPLC analysis using a Superose™ 6 Increase column

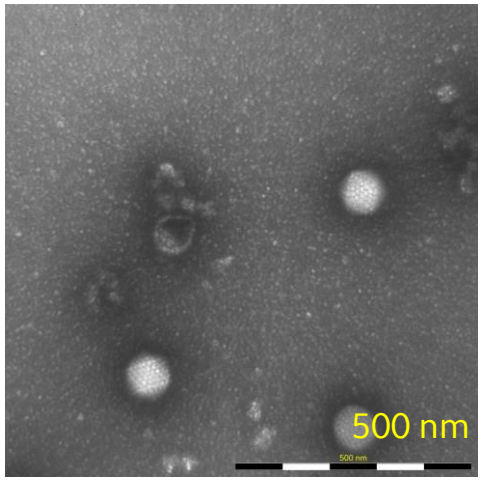


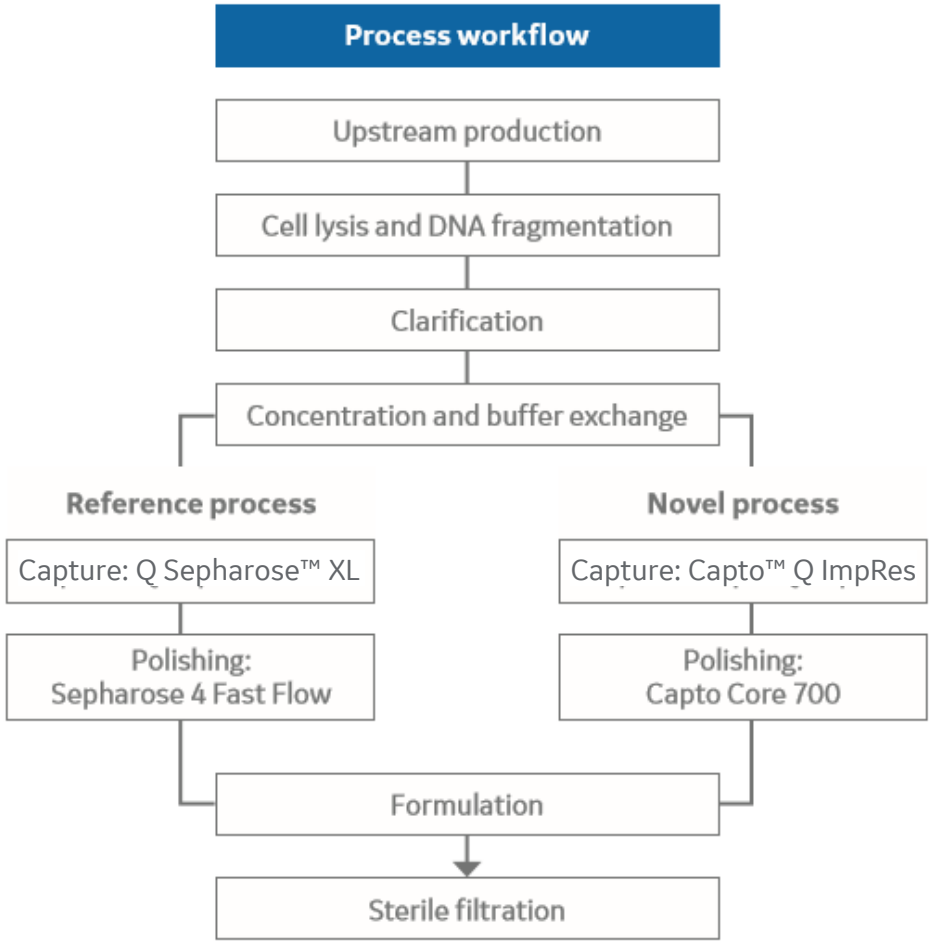
SEC= size exclusion chromatography
HPLC = High performance liquid chromatography

Novel process shows improved impurity reduction

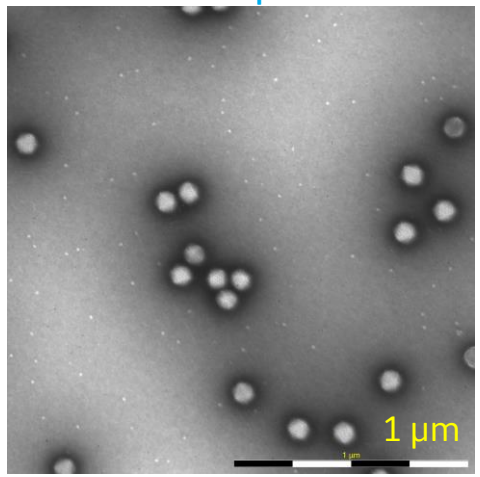
Reference process

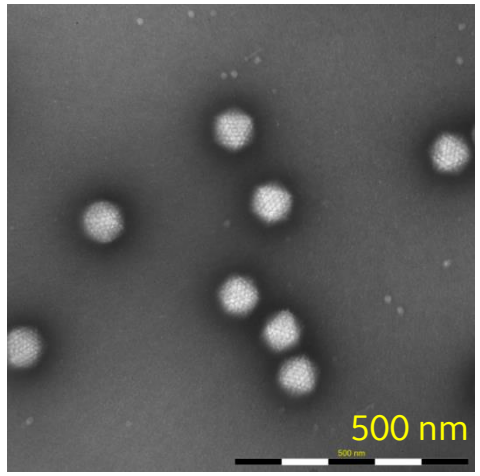






Novel process

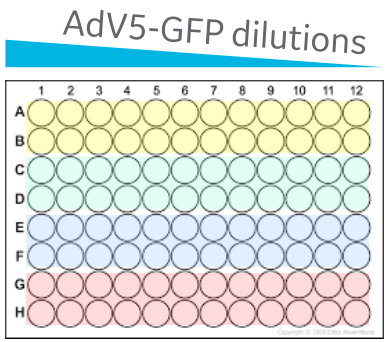




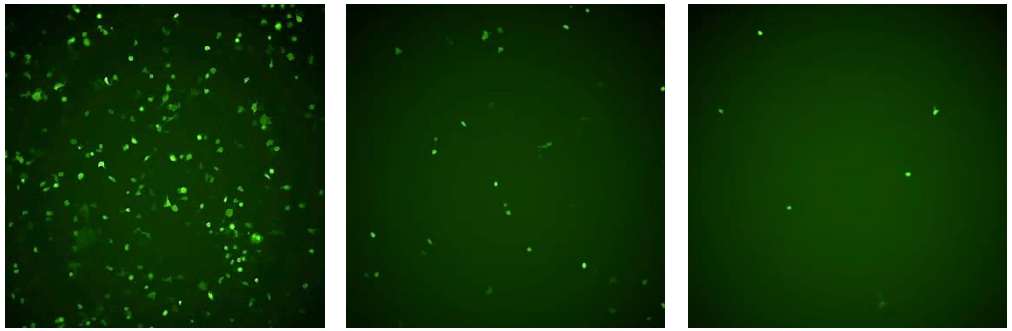
Transmission electron microscopy imaging performed by Vironova AB using MiniTEM™ system

Virus titer assay development

Adenovirus infectious virus titer with IN Cell Analyzer



42 hrs

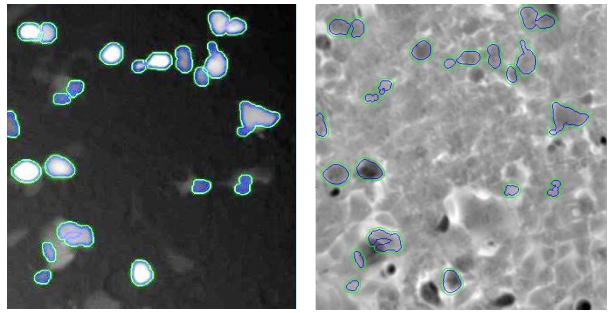


IN Cell Analyzer

Automated counting of GFP foci

Automated fluorescence microscopy:

- Similar setup as $TCID_{50}$
- Cells in 96-well plate
- Serial dilution of virus
- Require fewer replicates

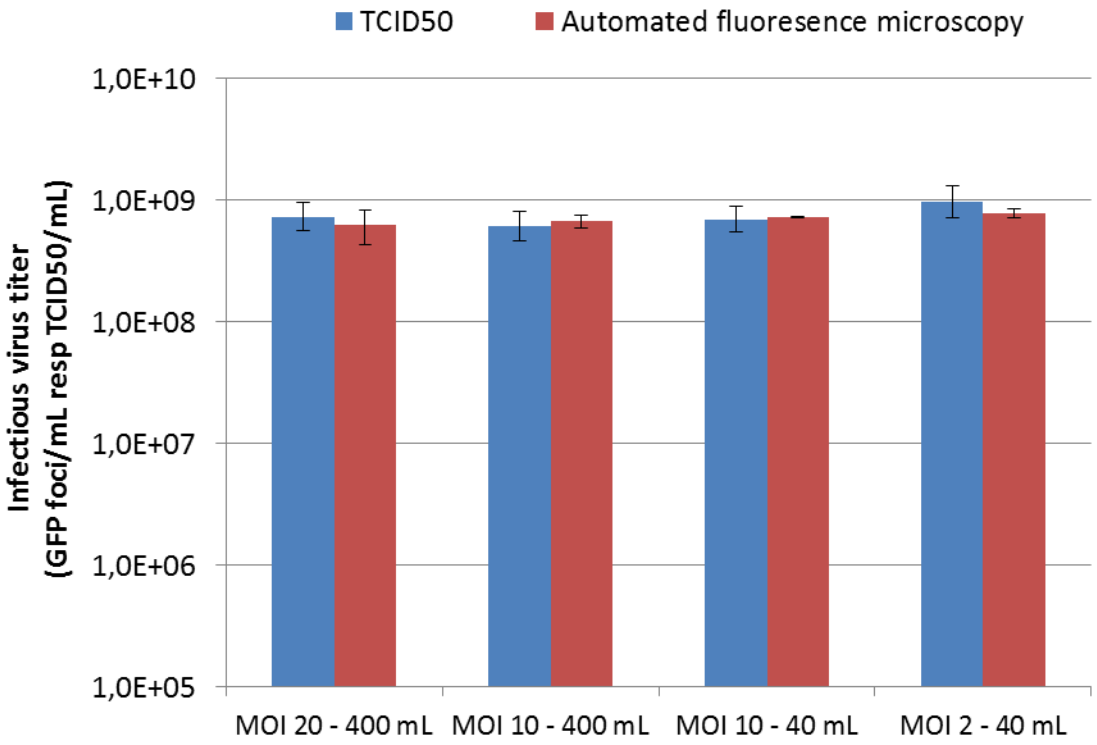


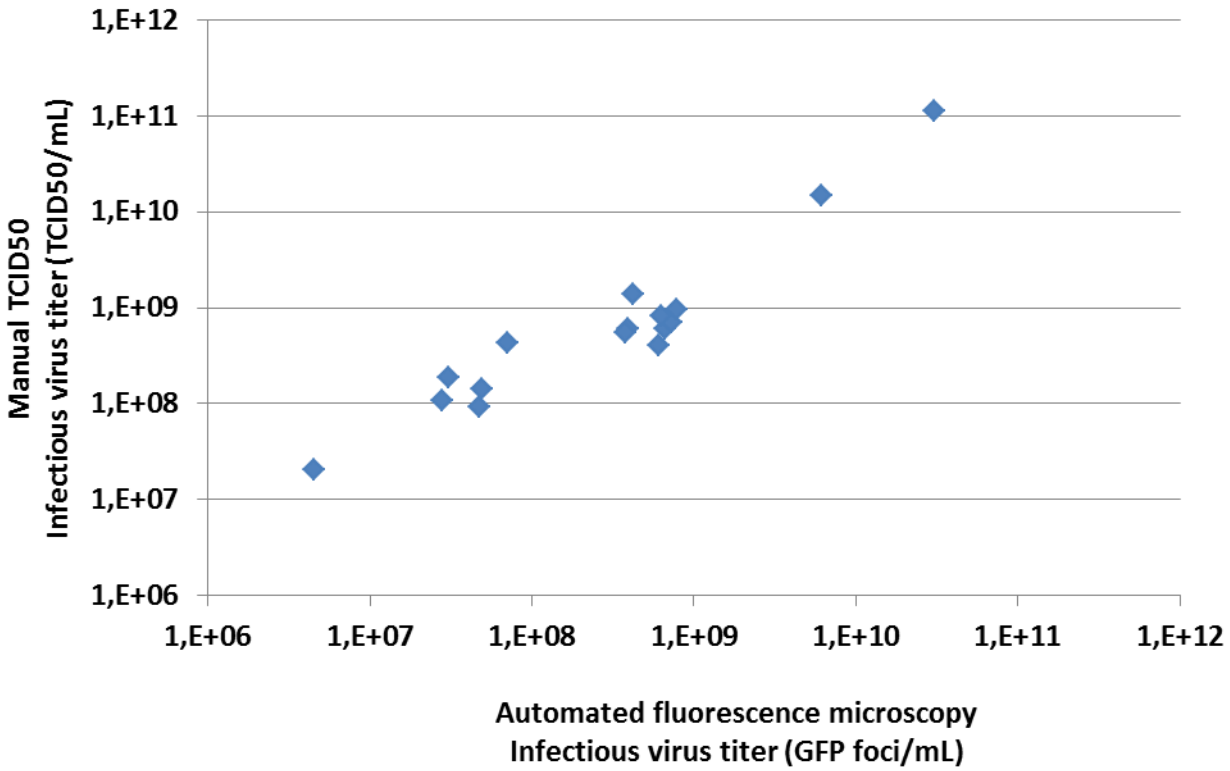
GFP Brightfield

Infectious virus titer (iVP/mL)

AdV5 = adenovirus serotype 5
GFP = green fluorescent protein
TCID = tissue culture infectious dose
iVP = infectious virus particles

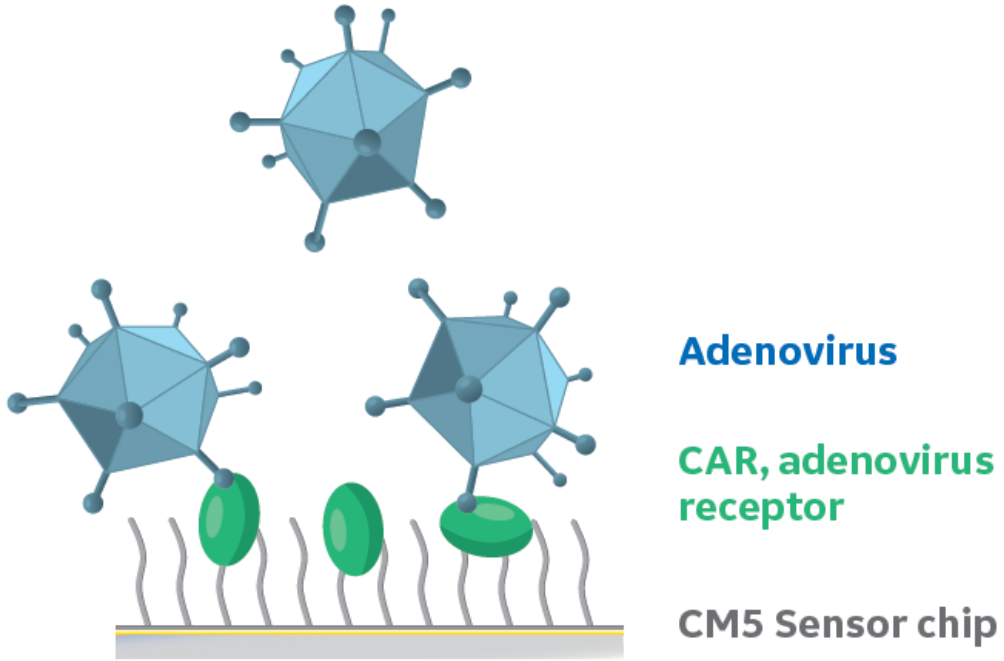
Good correlation between $TCID_{50}$ and automated fluoresence microscopy



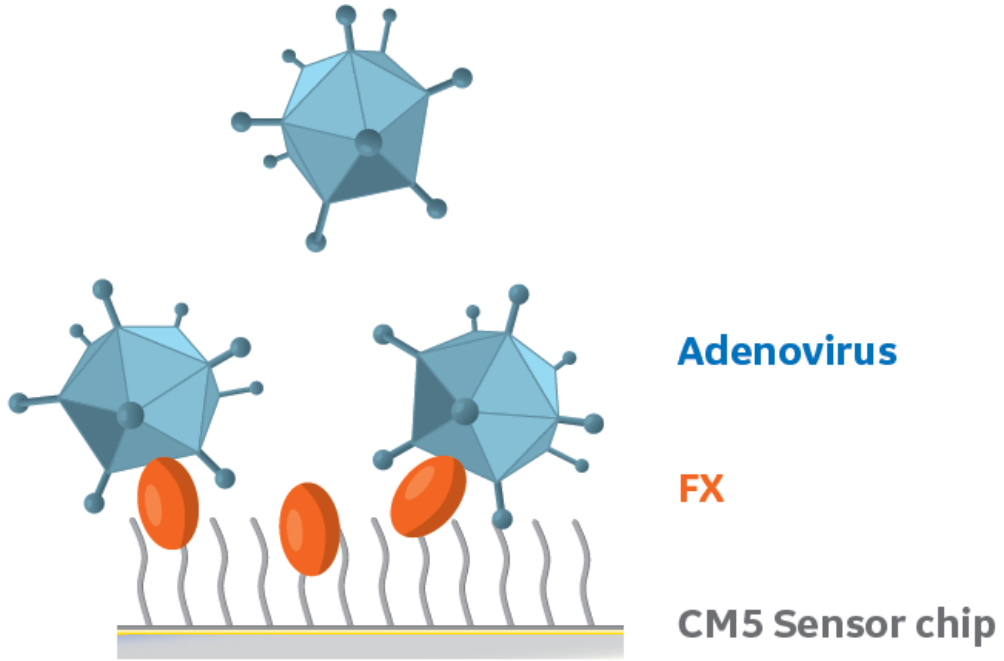


$TCID_{50}$ = tissue culture infectious dose
GFP = green fluorescent protein
MOI = multiplicity of infection

Adenovirus titer with Biacore™ T200 assays

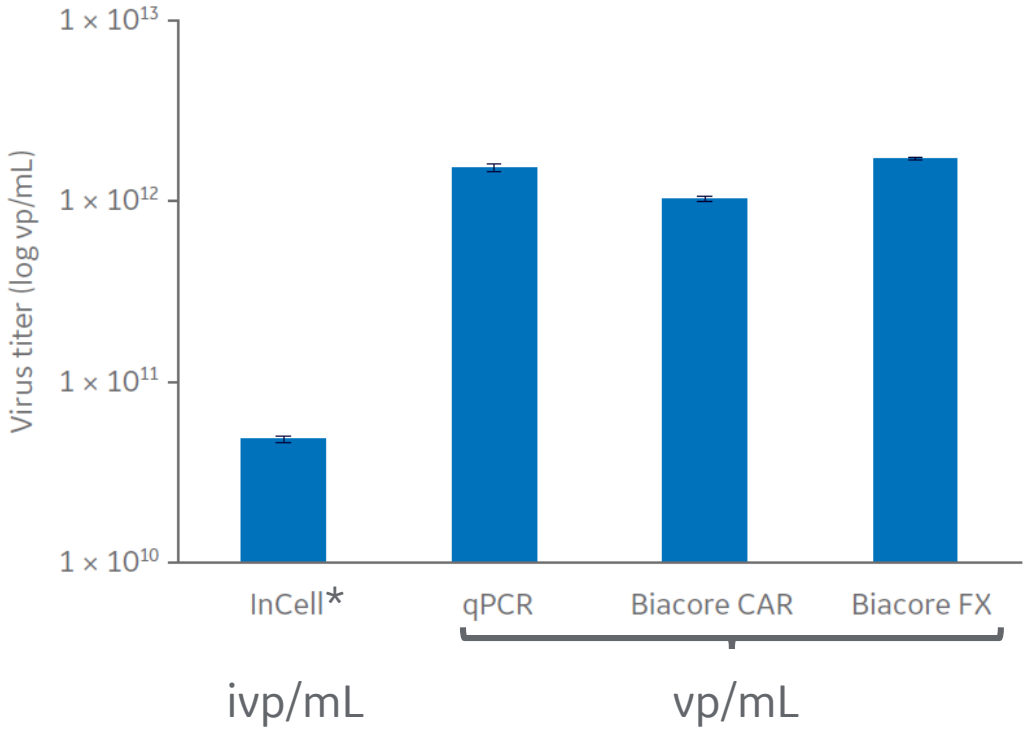


Coxsackie adenovirus receptor (CAR)



Factor X (FX)

Biacore™ T200 Adenovirus titer results are comparable to qPCR



* Infections virus titer (ivp/mL) is expected to be lower than total virus titer (vp/mL). Regulatory requirements for the ratio of total to infections virus particles is < 30 (FDA).

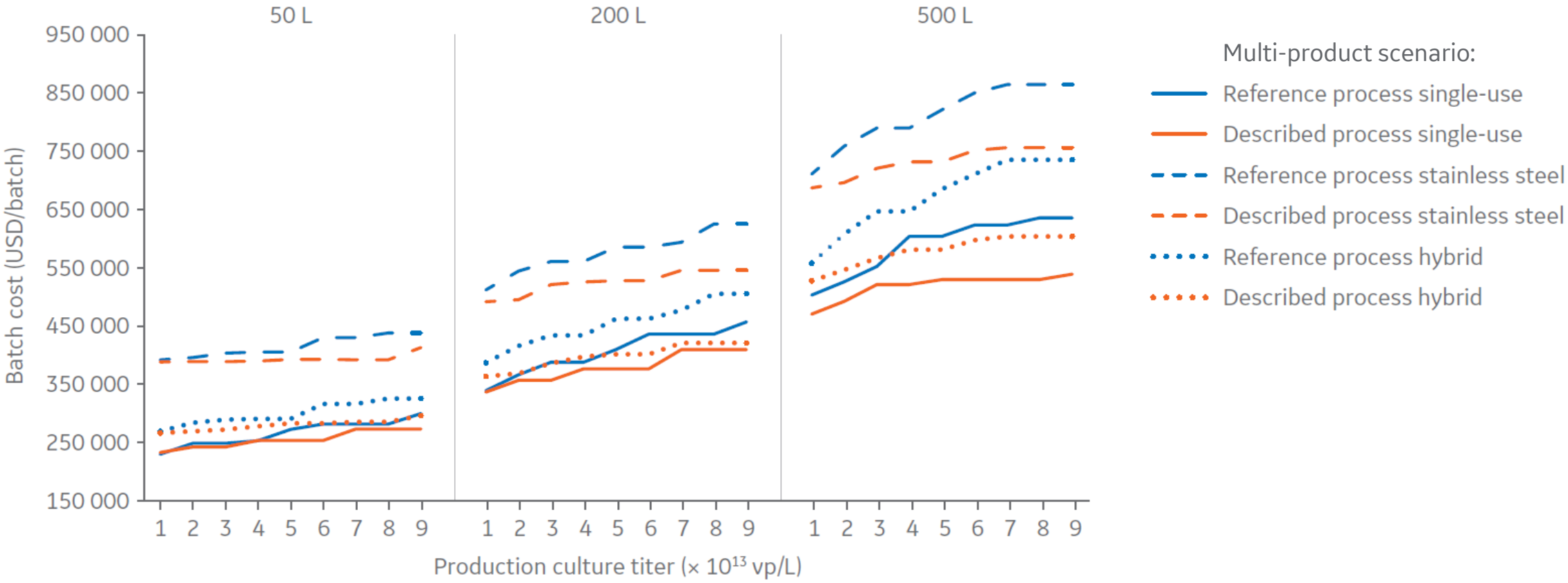
- Convenient assay
- Reproducible, CV < 5%
- Sensitive, sample dilutions (100 to 200-fold) reduce effect of buffer components
- Immobilized surface stable for at least one week

Process economy evaluation

Process economy simulation

- The cost per batch was compared between the novel resin process and the reference process
- Stainless steel and single-use equipment configurations were compared in different production scenarios (different scales, titers and number of batches per campaign)
- Single-use scenario compatible with large-scale GMP production (i.e. FlexFactory™ platform)

Cost per batch comparison: New process is favorable and scales well



Conclusion

Conclusions

- Viral vectors becoming important for vaccines, cell- and gene therapy
- Adenovirus process based on single-use bioreactors and scalable technology in chemically defined medium
- Modern purification technologies for scalable purification
- Fulfills regulatory requirements
- Process technology compatible with large-scale GMP production (i.e FlexFactory™ platform)

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