Perspectives from the CAACB on the Current Benefits and Challenges of NGS Adoption for Viral Safety Testing

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Virus contamination events have a large impact

- Patient impact
  - Potential patient safety issue
  - Product stock-out
- **Manufacturing impact**
  - Production shutdown
  - Comprehensive investigation required
  - Corrective and preventive actions
  - Manufacturing plant decontamination
- **Regulatory impact**
  - Delay in product approval
  - Exposes company to *intense* regulatory scrutiny
- **Business impact**
  - Lost product / sales
  - Changes public perception of product quality
  - Encourages competition
  - Complicates partnerships
  - Exposes company to lawsuits
  - Diverts focus of company leadership

There is a lack of industry wide knowledge

- Many companies **have not publicly disclosed** virus contamination events
  - No obligation to disclose unless the contamination results in a “material change” to the business
  - Motivated by concerns for negative publicity
- Companies that have disclosed **rarely describe the event in sufficient detail** to be of significant value
- Companies are only really **able to learn from their own contamination** events

MIT started the CAACB to fill this information gap

CAACB

Consortium on Adventitious Agent Contamination in Biomanufacturing

Biopharmaceutical industry consortium housed at MIT Center for Biomedical Innovation launched in 2011

Goals of the CAACB

- **Share experiences** in the control and risk mitigation of adventitious agent contamination in biomanufacturing
- Provide a forum for companies to **network**
- Identify **best industry practices**
- Provide opportunities to **benchmark**
- Conduct **collaborative research** activities
- Promote generation and application of **new technologies**
- Provide members **early access to data and participation in analyses**

Key CAACB activities

CAACB Workshops

- Interactive meetings with presentations, case studies and open discussion

CAACB Member Driven Projects

- Collection and Analysis of Virus Contamination Data in Biomanufacturing
- Evaluation of Media Treatment Options*
- Handbook for Risk Assessment and Risk Control of Virus Contamination in Biomanufacturing*
- Facility Segregation for Viral Safety*
- Risk Assessment of a Model Biomanufacturing Process*
- Collaboration to Address Emerging Virus / Pandemic Preparedness
- Historical Evaluation of the *In Vivo* Adventitious Virus Test and its Potential for Replacement with NGS*
- Collaboration to Modernize CHO Virus Safety Testing and Establish a Virus Risk Assessment Framework‡

* Completed projects
‡ New in 2022

Thank you to our CAACB Member Companies

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Premise of inaugural project – collective intelligence is more valuable than individual experience

The **confidential collection** of industry-wide viral contamination data and a subsequent risk analysis assessment **would be a highly valuable "lessons learned" exercise** for industry and could guide companies in best practices to mitigate the risks that lead to these events.

Scope of the Virus Contamination in Biomanufacturing Study

Included

- Mammalian cell culture
- rDNA biopharmaceuticals and cell culture produced vaccines
- Manufacturing: R&D (Pilot plant and larger); Commercial
- Viruses
- CAACB members
- Drug sponsors and CMOs
- GMP and non-GMP
- Since 1980
- Worldwide

Excluded

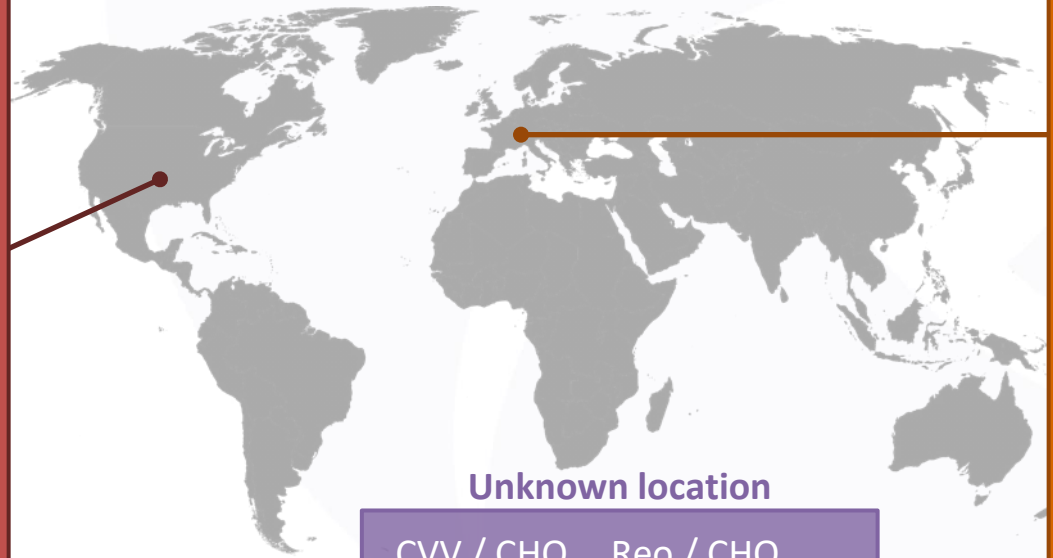
- Fermentation (e.g. bacteria)
- Vet products and egg produced vaccines
- Non-biomanufacturing (Discovery, QC labs)
- Mycoplasma, bacteria, fungi
- Non-members

The CAACB Virus Contamination in Biomanufacturing Survey

- 166 question comprehensive survey
- Covered 13 sections
  - Contamination Event/Detection
  - Virus “False Positives”
  - Virus Controls in place at the time of contamination
  - Extent of contamination
  - Source of contamination
  - Process Breach
  - Containment
  - Investigation Management
  - Decontamination
  - Corrective Actions / Discarded Product / Restart
  - Preventive Actions
  - Cost of Event / Lessons learned
  - Viral Contamination Risk Management

29 virus contamination events have been reported publicly and to the CAACB

CVV / CHO
MMV / CHO
MMV / CHO
H Adeno / HEK293
MMV / CHO
Reo / Hu 1º Kidney
BTV / CHO
MMV / CHO
MMV / CHO
MMV / CHO
MMV / CHO
Vesivirus / CHO
Vesivirus / CHO



CVV / CHO
EHDV / CHO
Herpes / 1º Monkey
Herpes / Vero
MMV / BHK-21
Parainfluenza / MRC5
PCV-1 / Vero
Reo3 / MRC5
S Adeno / 1º Monkey
Vesivirus / CHO
Vesivirus / CHO

Unknown location

CVV / CHO	Reo / CHO
CVV / CHO	MMV / BHK-21
	EHDV/ Unk.

Note: events in ***bold italics*** were reported to the CAACB; others reported publicly

New insights from the CAACB Virus Contamination in Biomanufacturing Survey

1. Virus contamination events
   - are rare (21 reported to the CAACB over 35 years) based on volume
   - but not rare on a per-company basis and were expensive

43% of respondents have experienced a virus contamination in cell culture operations

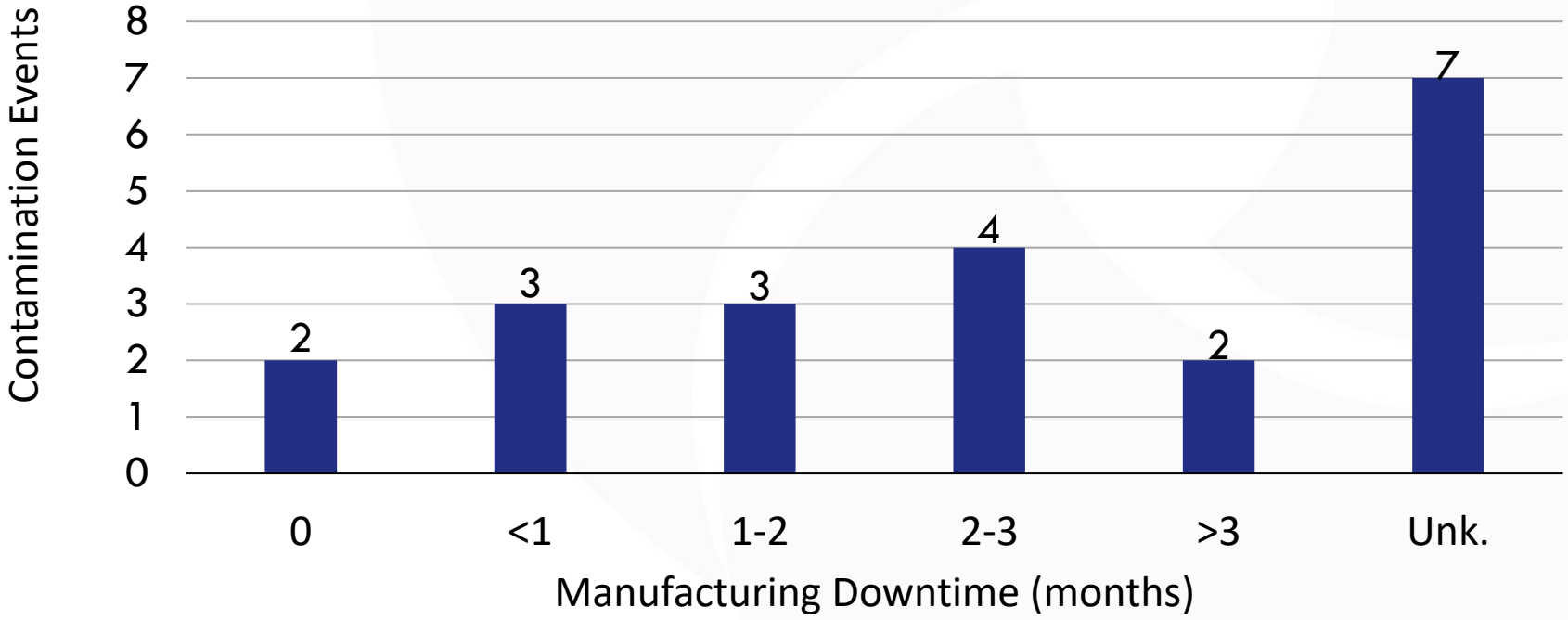
	Quantity
Companies completing survey *	23
Companies reporting a virus contamination	10
Virus contamination events reported (including R&D and GMP production)	21
Companies reporting a “false positive” event	16
“False positive” events reported	31

* Company Names Uncoupled from Data for Analysis

Dealing with a virus contamination event can be very expensive

Impact on Operations	Costs
Investigation	$1M – >50 M
Replacing discarded materials	$0.2M – 1M +
Decontamination	$0.2M - 1M+
Corrective actions	$0.2M – 2M+
Regulatory submissions	No data
Loss of revenue from product stock out	No data
Delays in product development; product approvals	No data
Product competitive disadvantage	No data
Decrease in company stock value	No data

The mean manufacturing plant downtime was 1 to 2 months



New insights from the CAACB Virus Contamination in Biomanufacturing Survey

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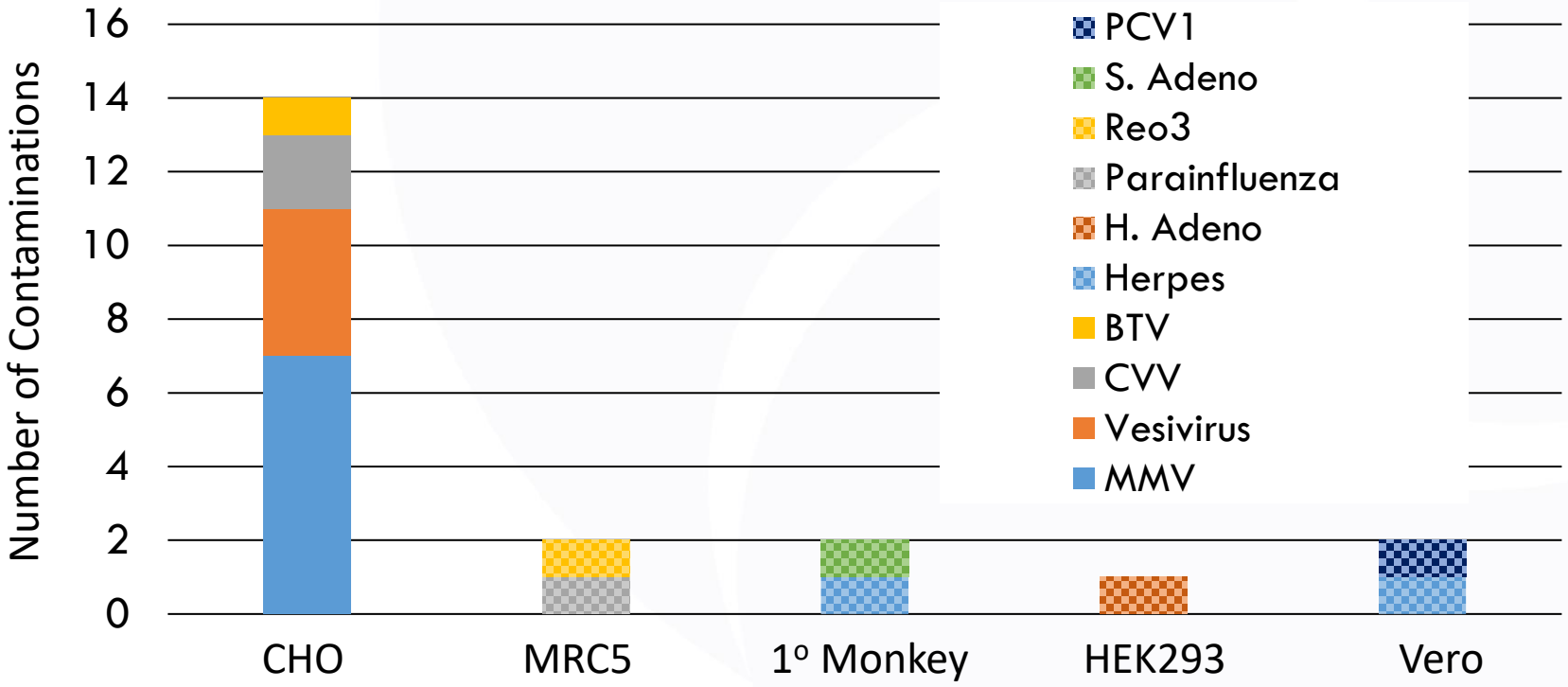
2. CHO cell cultures were contaminated by different viruses from different sources compared to human/primate cells

Ten different viral contaminants have been identified



Other publicly reported virus contaminants include EHDV.

Contaminations in human and primate cells came from different sources than in CHO cells

Contaminating Virus	Bovine Serum	Recombinant Media Component	Undetermined Media Component	Operator	Host Cell Line	Not Found
Vesivirus 2117	4					
Cache Valley Virus (CVV)	2					
Blue Tongue	1					
Mouse Minute Virus (MMV)		1	3			3
Herpes Virus				1		
Human Adenovirus Type 1				1		
Parainfluenza Virus Type 3				1		
Porcine Circovirus 1			1			
Reovirus Type 3				1		
Simian Adenovirus					1	

*Viruses in **black** were found in CHO cell lines.*
*Viruses in **red** were found in human or simian derived cell lines.*

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1. Virus contamination events
   - are rare (21 reported to the CAACB over 35 years) based on volume
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2. CHO cell cultures were contaminated by different viruses from different sources compared to human/primate cells
3. Virus safety testing has clear limitations, but when used in a targeted way can prevent virus spread in a facility

The *in vitro* virus test gave a *false negative* in five GMP contamination events

Test used for QC lot release	QC Test Positive	QC Test Negative
PCR	1*	0
In Vitro Virus Test	11**	5**
Immunofluorescence	2***	0

* In one event, PCR was positive but the IVV test was negative

** Two events found some bioreactors were positive by PCR, but negative by In Vitro Virus test, whereas other bioreactors were positive by both tests.

*** Two events used an In Vitro Virus test for QC lot release with an end-point immunofluorescent (IF) measurement; in both cases, the IF signal was positive and the CPE effect was negative.

NGS identified the virus in 7 contamination events

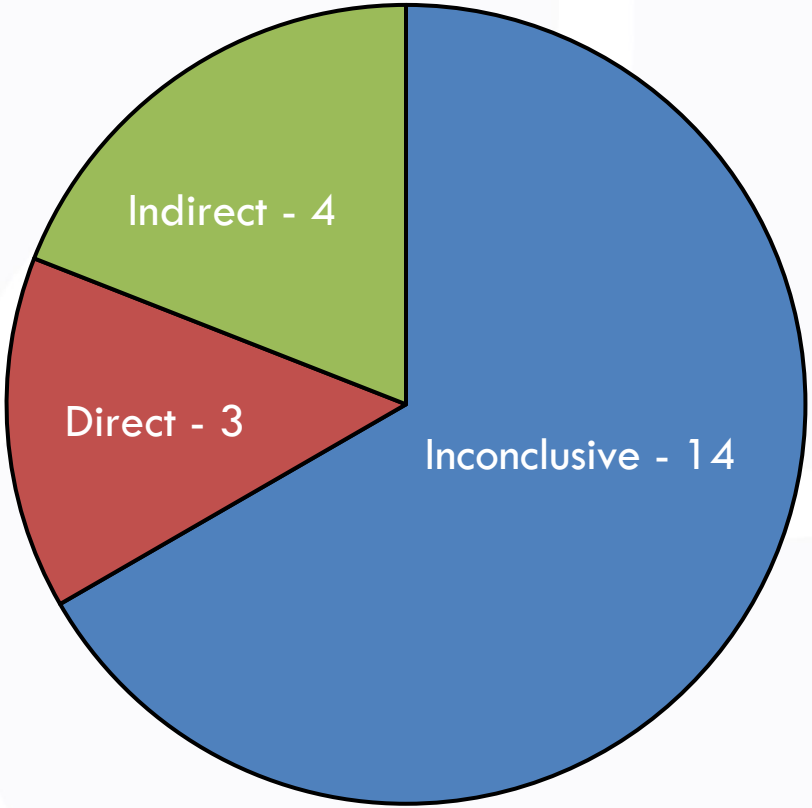
Method	Initial Detection	Confirmation	Virus Identification
Changes in Cell Culture Performance	11		
PCR	3	10	11
In Vitro Virus Test	5	9	
Electron Microscopy		4	3
Immunofluorescence	1	2	2
MS Protein Sequencing			2
NA Fingerprinting		1	1
Serology			6
NGS	1	1	7

The source of the contamination was directly identified in only 14% of cases

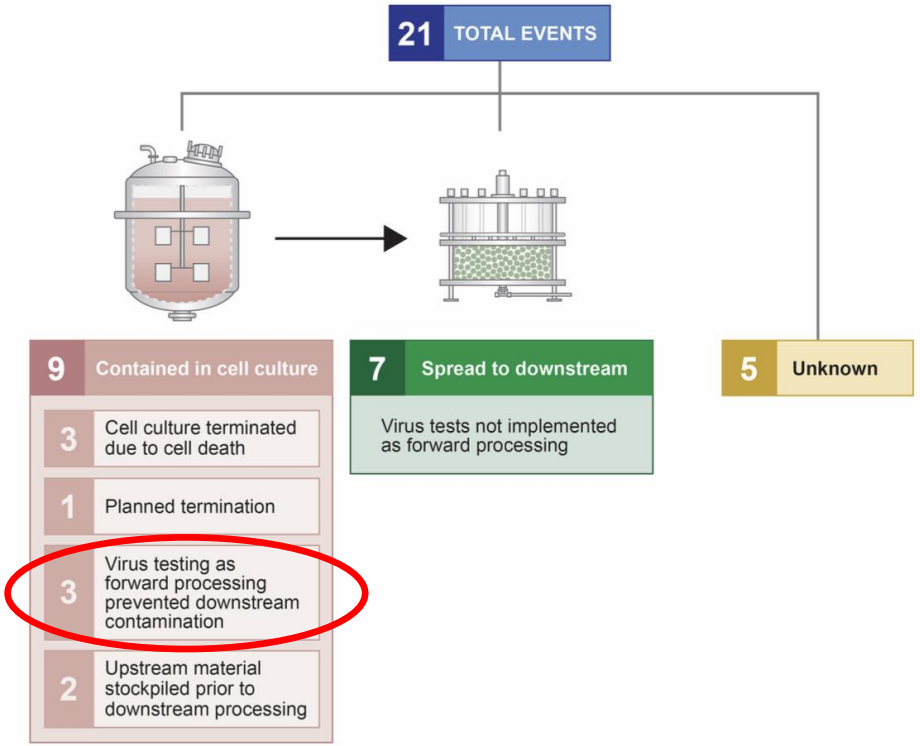
Direct detection and identification of the source of the contamination required either:

1. Biological amplification coupled to a PCR; or
2. Concentration of the raw material was necessary

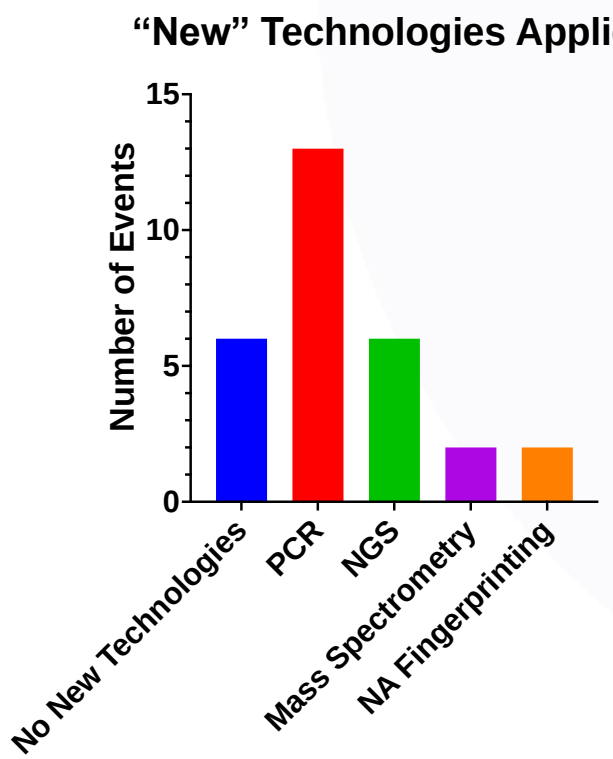
In all other cases *where tested*, suspected sources tested negative

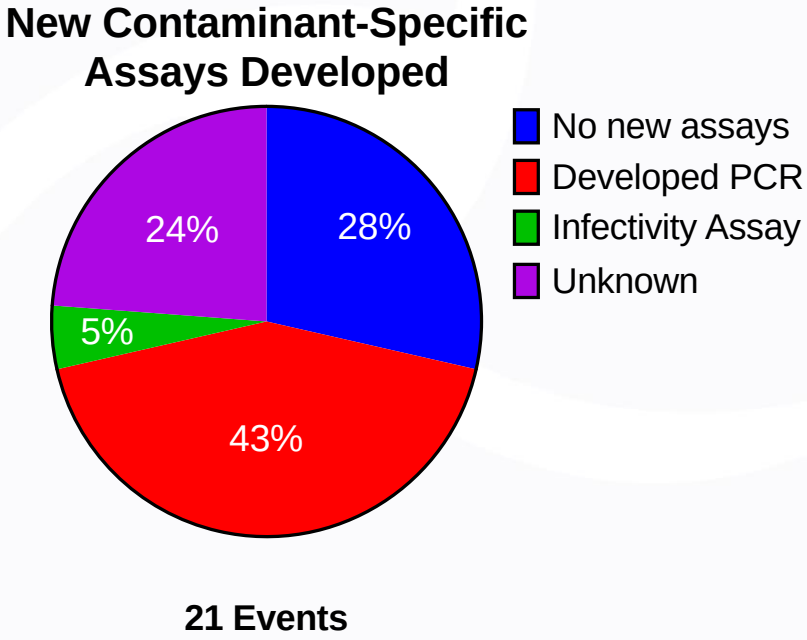


Rapid virus tests prevented forward processing in 3 contamination events

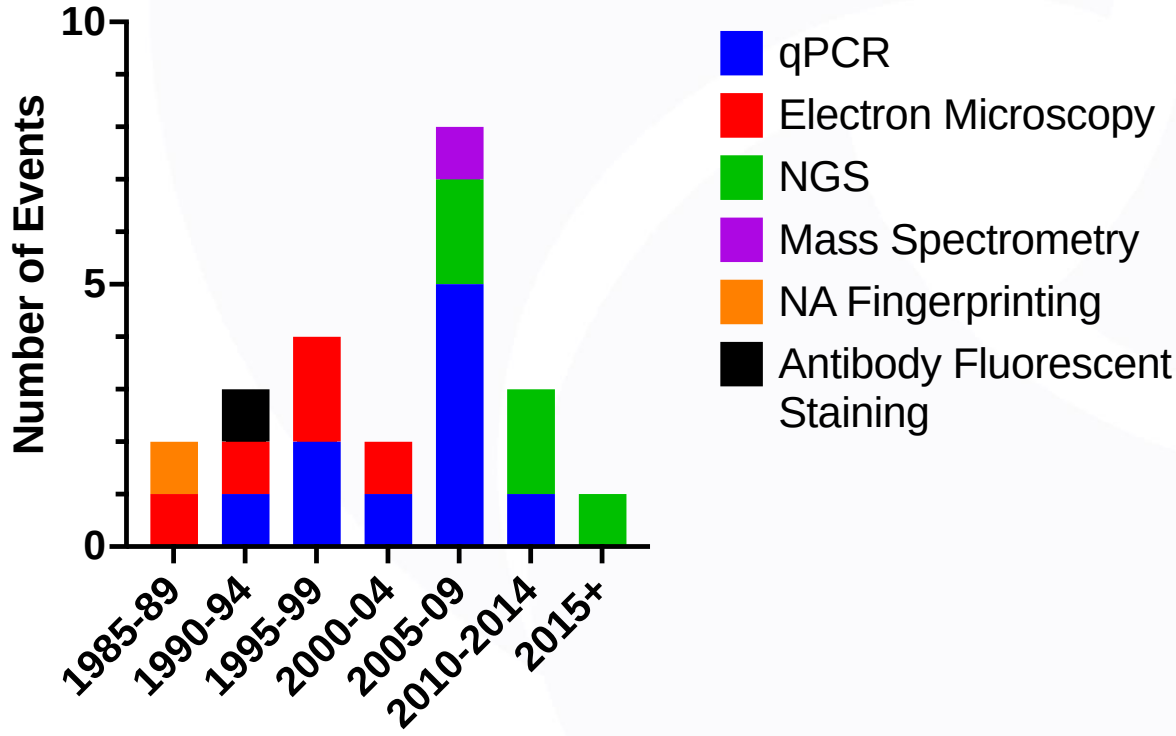


NGS and PCR were the main “new” technologies applied during the event investigation





CAACB members increasingly find NGS the most useful tool for viral contaminant identification



Key takeaways from the Virus Contamination in Biomanufacturing Project

- The frequency of virus contamination is *extremely low*.
- Viruses contaminating human and primate cells ***were attributed primarily to human sources***, e.g., from manufacturing operators
- While cell culture performance was the most frequent initial indication of a contamination, in *two events, performance did not change.*
- The *in vitro virus test is the gold standard* for broad spectrum viral contaminant detection, but *it is lengthy (14-28 days) and in 5 contamination events did not detect the contaminating virus.*
- Rapid, in-process virus detection methods (PCR) have been used to contain the contamination in bioreactors.
- During the investigations, ***testing of raw materials were positive in only 3 of 11 events*** and those positive results required either biological amplification (2 events) or concentration (1 event).
- Large-scale downstream viral clearance was effective at removing viral contaminants.

Read more about this work in Nature Biotechnology (does not include additional data presented here)

Barone, PW et al. Viral Contamination in Biologic Manufacture and Implications for Emerging Therapies. Nat. Biotech.; 2020 38:563-572.

nature biotechnology

PERSPECTIVE

https://doi.org/10.1038/s41587-020-0507-2

Check for updates

Viral contamination in biologic manufacture and implications for emerging therapies

Paul W. Barone[1], Michael E. Wiebe[1], James C. Leung[1], Islam T. M. Hussein[1], Flora J. Keumurian[1], James Bouressa[2,25], Audrey Brussel[3,26], Dayue Chen[4,27], Ming Chong[5], Houman Dehghani[6,28], Lionel Gerentes[7], James Gilbert[8,29], Dan Gold[9], Robert Kiss[10,30], Thomas R. Kreil[11], René Labatut[3], Yuling Li[12,31], Jürgen Müllberg[13], Laurent Mallet[7,32], Christian Menzel[14], Mark Moody[15,33], Serge Monpoeho[16], Marie Murphy[4], Mark Plavsic[17,34], Nathan J. Roth[18], David Roush[19], Michael Ruffing[20], Richard Schicho[21,35], Richard Snyder[22], Daniel Stark[23], Chun Zhang[24,36], Jacqueline Wolfrum[1], Anthony J. Sinskey[1] and Stacy L. Springs[1]✉

Recombinant protein therapeutics, vaccines, and plasma products have a long record of safety. However, the use of cell culture to produce recombinant proteins is still susceptible to contamination with viruses. These contaminations cost millions of dollars to recover from, can lead to patients not receiving therapies, and are very rare, which makes learning from past events difficult. A consortium of biotech companies, together with the Massachusetts Institute of Technology, has convened to collect data on these events. This industry-wide study provides insights into the most common viral contaminants, the source of those contaminants, the cell lines affected, corrective actions, as well as the impact of such events. These results have implications for the safe and effective production of not just current products, but also emerging cell and gene therapies which have shown much therapeutic promise.

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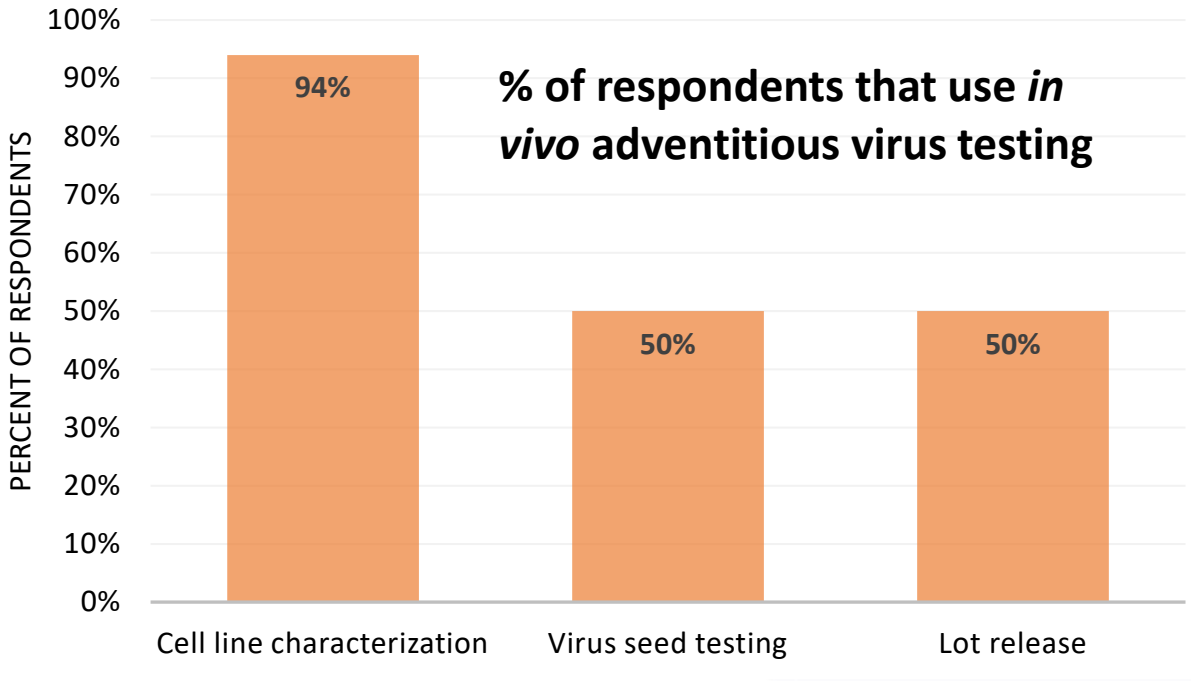
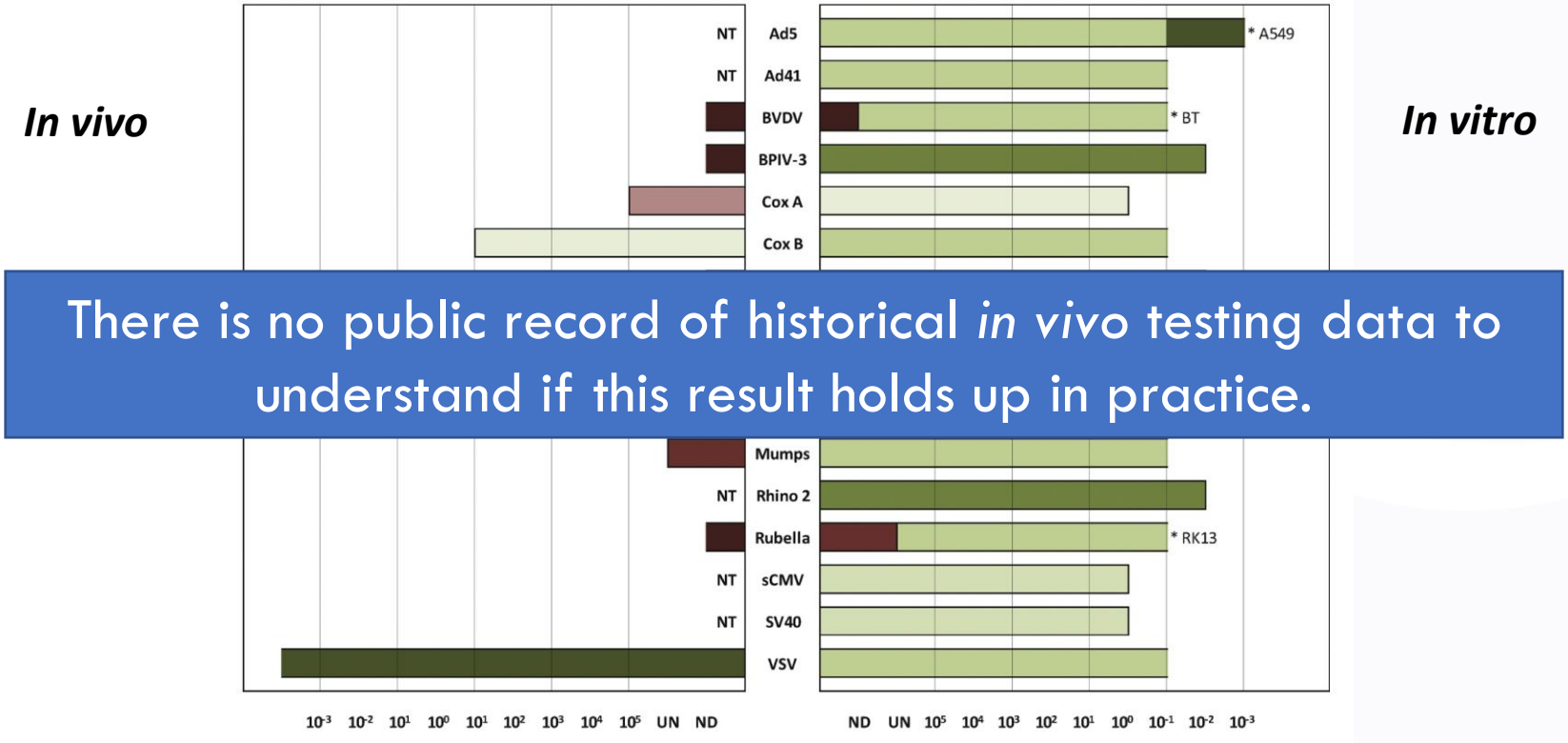
CAACB members report continued use of *in vivo* virus test



Since 2000 CAACB members have performed:

- **More than 10,000** ***in vivo*** adventitious virus tests (using >84,000 animals)
- **More than 67,000** ***in vitro*** virus tests

What value does the *in vivo* adventitious virus test add?



There is no public record of historical *in vivo* testing data to understand if this result holds up in practice.

J. Gombold et al. *Vaccine* 32 (**2014**) 2916-2926

Premise of the CAACB work

There is little publicly available information to guide biopharmaceutical manufacturers in assessing when and in what situations it may be appropriate to replace the existing broad spectrum *in vivo* or *in vitro* virus tests with an alternative method.

To address this gap, **the CAACB has collected historical data** from 20 biopharmaceutical industry members**, on their experience with the *in vivo* adventitious virus test, the *in vitro* virus test, and the use of high-throughput sequencing for viral safety.**

What value does the *in vivo* adventitious virus test add?

CAACB members were asked if, over the lifetime of their use of the *in vivo* adventitious virus test, **they had experienced a positive in vivo adventitious assay test that was NOT also detected in another supporting assay**

→ **This has not happened!**

How reliable are the *in vivo* and *in vitro* virus tests?

	In vivo adventitious virus test	*In vitro* virus test
Number of tests	>10,000	>67,000

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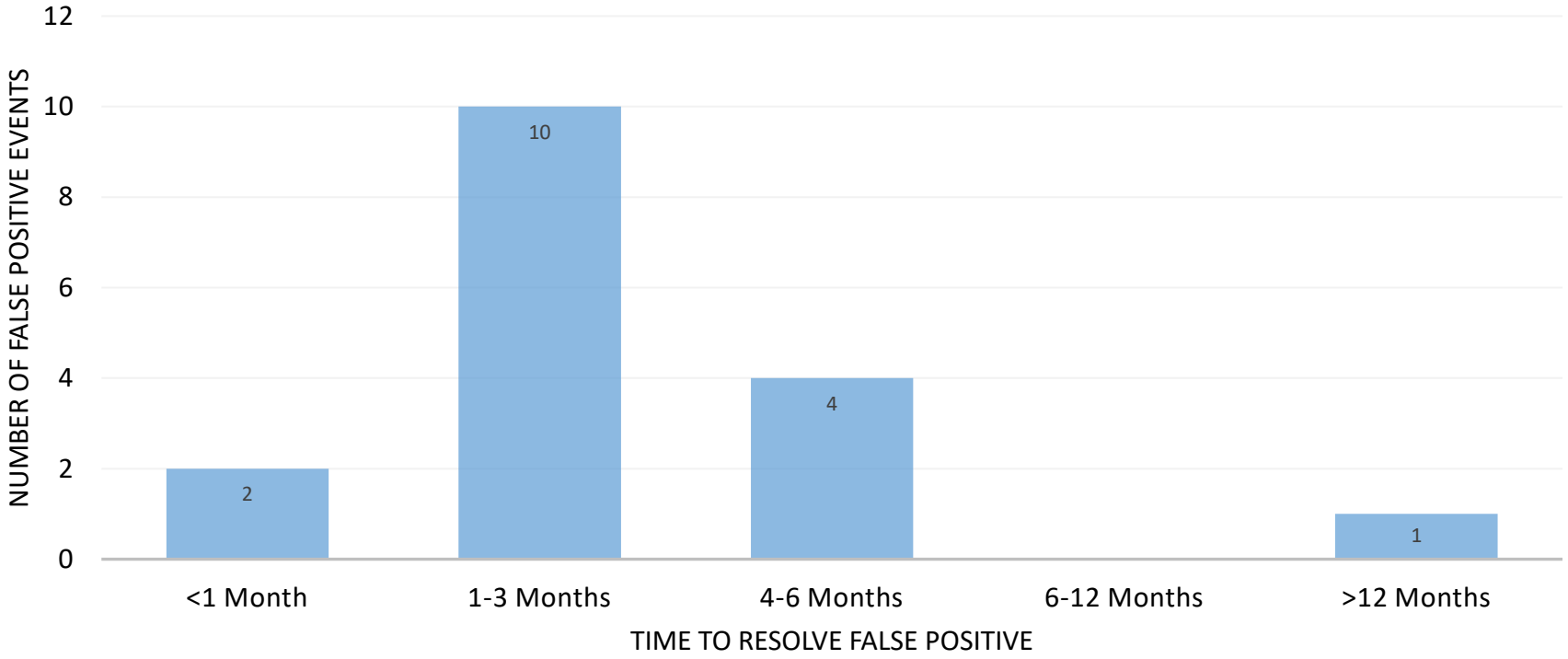
	Adult Mice	Suckling Mice	Eggs	Guinea Pigs	HAP	MAP	RAP
False Positives	2	4	5	0	2	7	0

In vivo false positive events took months to resolve



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Repeats (# / rate)	> 21 / 0.2%	NA

Repeat assays take time (18 days to months):

- 37% of repeats took less than 1 month
- 63% of repeats took 1 – 3 months

Our conclusion: alternatives are needed for the *in vivo* adventitious virus test

Alternatives are needed for the *in vivo* adventitious virus test

1. The *in vivo* adventitious virus test has not been reported to detect a viral contaminant that was not also detected in another assay;
2. More than 84,000 animals have been used by CAACB member companies for *in vivo* testing;
3. There are biologic products for which *in vivo*, or *in vitro*, testing will not work (e.g., viral products with no neutralizing antibody)

Sequencing is a potential alternative for viral detection

- Short-read sequencing has been used to detect previously undetected viral contaminants in biologic manufacturing and cell lines.
- Sequencing of detected PCV-1 contamination in 2010.[1]
  - 41% of viral reads in Rotarix™ were attributed to PCV-1
  - Rotarix™ had been given to 32,000 infants in pre-licensure studies.
  - The contaminating PCV-1 had been present since the early stages of Rotarix™ development.

1.Victoria, J. G., *et al*. Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus. *J. Virol.* (2010).
2. Ma, H., *et. al.* Identification of a Novel Rhabdovirus in Spodoptera frugiperda Cell Lines. *J. Virol.* (2014)

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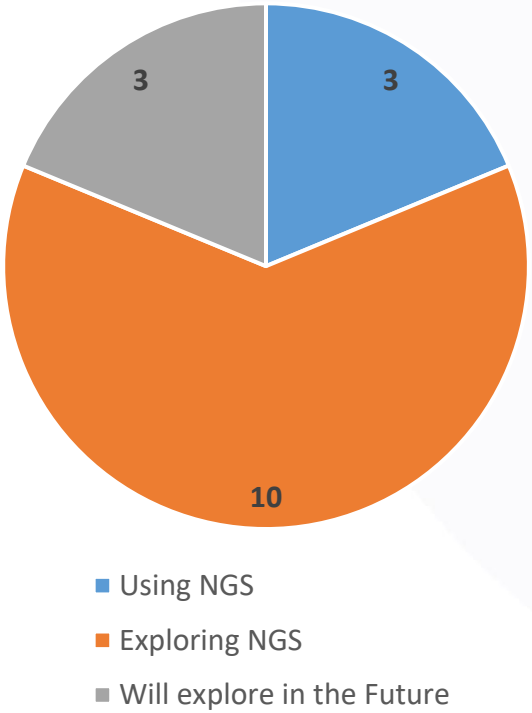
- Sequencing of detected PCV-1 contamination in 2010.[1]
- Sequencing has detected a novel rhabdovirus in insect cells[2]
  - Baculovirus-insect cell expression systems have been used for licensed viral vaccine production.
  - Previously undiscovered rhabdovirus in the cells themselves.

1.Victoria, J. G., *et al*. Viral Nucleic Acids in Live-Attenuated Vaccines: Detection of Minority Variants and an Adventitious Virus. *J. Virol.* (2010).
2. Ma, H., *et. al.* Identification of a Novel Rhabdovirus in Spodoptera frugiperda Cell Lines. *J. Virol.* (2014)

The regulators agree!

- **ICH Q5A (R2)** guidelines for viral safety evaluation in biotechnology products
  - Released in June 2024
  - "Non-targeted NGS is encouraged as a replacement for *in vivo* assays due to its breadth and sensitivity of virus detection and the limitations of the *in vivo assays*"
- **Eur. Ph. 2.6.41** draft chapter
  - Commentary closed in July 2024
  - Outlines methodologies used for detection of viral extraneous agents in biological products

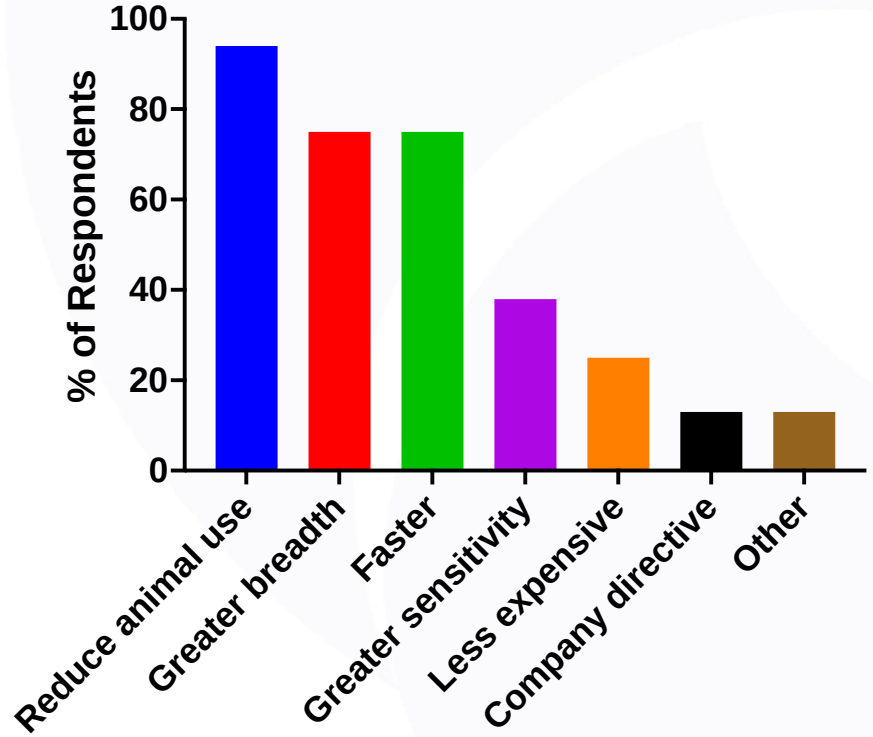
CAACB member companies demonstrate a clear interest in NGS as an alternative method



Companies are interested in replacing or supplementing existing assays:

- 69% of respondents are exploring replacing *in vivo* with NGS
- 50% of respondents are exploring supplementing *in vivo* with NGS
- Some companies have included NGS in IND filings

Motivation for replacing or supplementing existing tests with NGS



Technical challenges to NGS implementation

- Sample isolation and handling
  - PDA AVDTWG has on-going and published work to create industry consensus on this effort
  - Eur. Ph. 2.6.41 details sample handling
- Bioinformatics requires in-house expertise and there is a lack of guidance on setting thresholds for algorithms
  - CROs are filling this need for many clients
- Lack of standards
  - WHO Reference Standards for adventitious virus detection now available
- Analysis time may be too long for some product modalities

NGS is a critical tool for viral safety control

- Developing an NGS assay takes time
  - Starting this now will save critical time should an event occur
- Broad spectrum detection that can be operated in an agnostic manner
  - Detected previously undetected viral contaminants
- Comparable or better sensitivity than the *in vitro* and *in vivo* adventitious virus test (when compared to viruses previously tested *in vivo*)
- Simultaneous detection and identification of contaminant
- NGS takes less time than the *in vitro* and *in vivo* test

Read more about this work in Biologicals

Barone, P. W. *et al*. Historical evaluation of the *in vivo* adventitious virus test and its potential for replacement with next generation sequencing (NGS). Biologicals 101661 (2023) doi:10.1016/j.biologicals.2022.11.003.

Biologicals 81 (2023) 101661

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Historical evaluation of the *in vivo* adventitious virus test and its potential for replacement with next generation sequencing (NGS)

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ABSTRACT

The Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) collected historical data from 20 biopharmaceutical industry members on their experience with the *in vivo* adventitious virus test, the *in*

Acknowledgements

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Dr. James Leung – Lead Investigator

Dr. Michael Wiebe – Lead Investigator

Relevant publications

1. Barone, PW *et al.* Viral Contamination in Biologic Manufacture and Implications for Emerging Therapies. Nat. Biotechnol; **2020** 38:563-572.
2. Barone, P. W. *et al*. Historical evaluation of the in vivo adventitious virus test and its potential for replacement with next generation sequencing (NGS). Biologicals 101661 (**2023**) doi:10.1016/j.biologicals.2022.11.003.
3. Barone, PW, *et al.* Biopharmaceutical Industry Approaches to Facility Segregation for Viral Safety. PDA J Pharm Sci Technol; **2019** 73:191-203.
4. Barone, PW, *et al.* Quality Risk Management in the Context of Viral Contamination *in Microbiological Identifications: Keys to a Sustainable Program edited* by Dona Reber and Mary Griffin (**2018**).

Thank You!

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