HTS and the future of vaccine analytics

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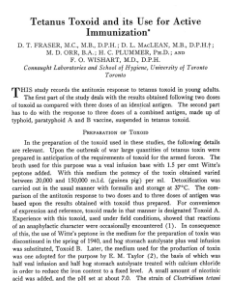
Vaccines – past, present, and future

The Past


Tetanus Toxoid and its Use for Active Immunization*

D. T. FRASER, M.C., M.B., D.P.H.; D. L. MacLEAN, M.B., D.P.H.†; M. D. ORR, B.A.; H. C. PLUMMER, Ph.D.; and F. O. WISHART, M.D., D.P.H.

Connaught Laboratories and School of Hygiene, University of Toronto, Toronto

THIS study records the antitoxin response to tetanus toxoid in young adults. The first part of the study deals with the results obtained following two doses of toxoid as compared with three doses of an identical antigen. The second part has to do with the response to three doses of a combined antigen, made up of typhoid, paratyphoid A and B vaccine, suspended in tetanus toxoid.

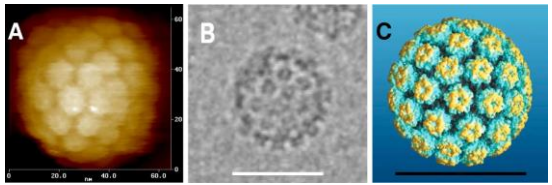
PREPARATION OF TOXOID

In the preparation of the toxoid used in these studies, the following details are relevant. Upon the outbreak of war large quantities of tetanus toxin were prepared in anticipation of the requirements of toxoid for the armed forces. The broth used for this purpose was a veal infusion base with 1.5 per cent Witte's peptone added. With this medium the potency of the toxin obtained varied between 20,000 and 150,000 m.l.d. (guinea pig) per ml. Detoxification was carried out in the usual manner with formalin and storage at 37°C. The comparison of the antitoxin response to two doses and to three doses of antigen was based upon the results obtained with toxoid thus prepared. For convenience of expression and reference, toxoid made in that manner is designated Toxoid A. Experience with this toxoid, used under field conditions, showed that reactions of an anaphylactic character were occasionally encountered (1). In consequence of this, the use of Witte's peptone in the medium for the preparation of toxin was discontinued in the spring of 1940, and hog stomach autolysate plus veal infusion was substituted, Toxoid B. Later, the medium used for the production of toxin was one adopted for the purpose by E. M. Taylor (2), the basis of which was half veal infusion and half hog stomach autolysate treated with calcium chloride in order to reduce the iron content to a fixed level. A small amount of ricinleic acid was added, and the pH set at about 7.0. The strain of *Clostridium tetani*

https://connaught.research.utoronto.ca/history/

- Live or killed whole-cell organisms
- Antigens non- or partially purified
- Al-based adjuvants only
- Basic chemical methods
- Potency & safety based on animal models
- “The product is the process”

The Present



Zhao et al (2021) Virology J. 9:52

- Recombinant antigens
- Engineered viruses
- “Well characterized” products
- 3Rs initiatives
- Process monitoring
- Modern analytics (immunochemistry, biophysics)
- Novel adjuvants & presentations
- Complex Regulatory environment

The Future

- New platforms (i.e. mRNA)
- Other impacts of COVID-19
- Advanced analytics
- “Factories of the Future”
- Other ??

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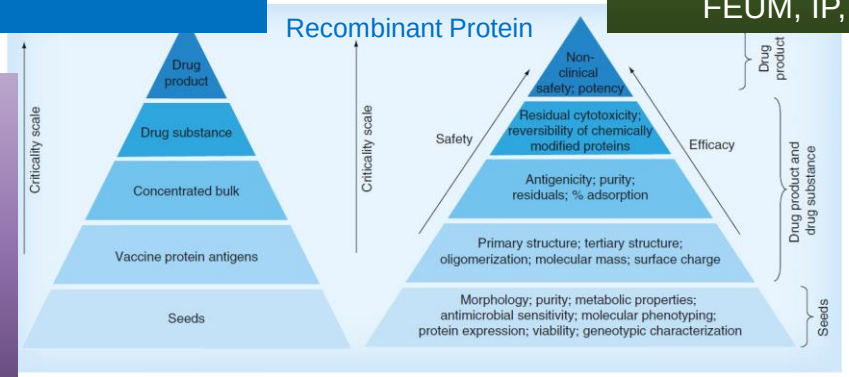
Vaccine analytics

- **Product drivers:**
  - Process development, evolution & scale-up
  - Biocomparability
  - Testing for characterization, release, stability & investigations
  - Product Analytical Life Cycle Management (ALCM)

- **Regulatory Drivers:**
  - ICH guidelines
  - WHO guidelines, monographs
  - FDA/CBER, EMA, China, Japan, UK, India, Mexico, Canada, Russia, Brazil, etc. etc.
  - Pharmacopoeia (USP, Ph.Eur., ChP, JP, BP, FEUM, IP, RP, etc.)

- **Product stages:**
  - Seeds
  - Upstream process
  - Downstream process
  - Drug Substance
  - Formulation
  - Drug Product



gure 1. Comparability based on product stages and product attributes.

Pharmaceutical Bioprocessing (2013) 1(4), 373–380

- **Product attributes:**
  - Safety
  - Potency
  - Purity
  - Structure
  - Stability

- **Analytics for product attributes that will become Critical Quality Attributes and becoming product specification**

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Vaccine Safety Testing

The Present

Still many animal-based safety tests and some *in vitro* microbiological tests that are inefficient and/or use animal-sourced reagents. Many of these tests are Compendial

The Future

Fully *in vitro* safety tests with no animal-derived reagents, accepted by global Health Authorities and described in relevant Pharmacopoeia

Adventitious Virus Detection by High Throughput Sequencing

- Highly sensitive, replaces multiple *in vivo* tests
- Ability to detect viral contaminant

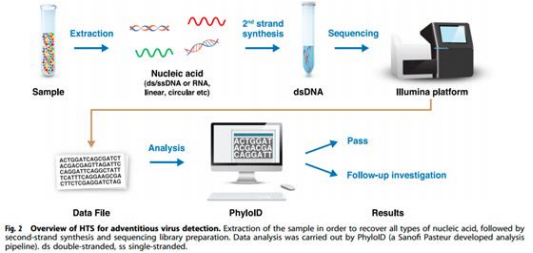
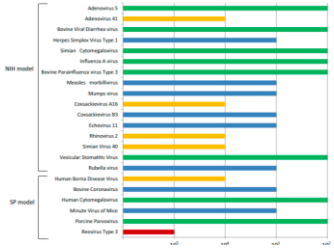


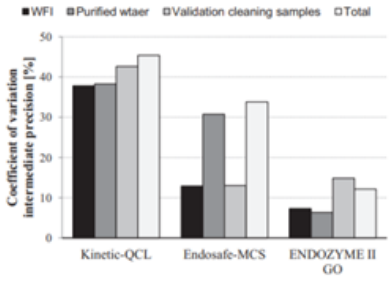
Fig. 2 **Overview of HTS for adventitious virus detection.** Extraction of the sample in order to recover all types of nucleic acid, followed by second-strand synthesis and sequencing library preparation. Data analysis was carried out by PhyloID (a Sanofi Pasteur developed analysis pipeline). ds double-stranded, ss single-stranded.

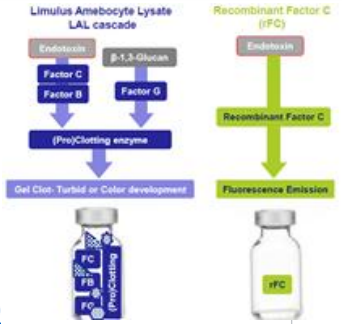


Charlebois et al., *NPJ Vaccines*, 2020

Alternative Endotoxin assay based on Recombinant Factor C

- Widely used LAL test is labor-intensive and uses animal-derived reagent (native FC from horseshoe crabs)
- Alternative rFC-based assay is animal-component free, more efficient, and less susceptible to interference





Marius et al., *Biologicals* 2020, 67:49

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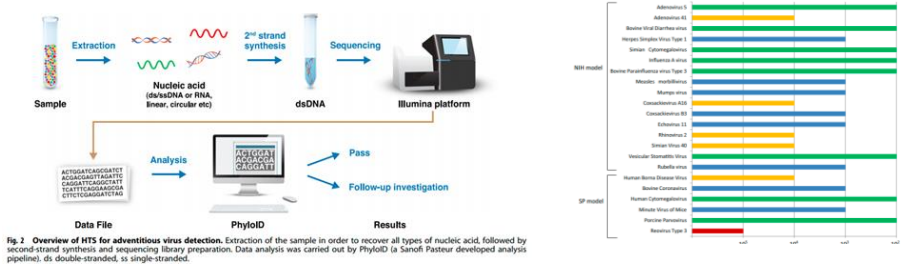

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Charlebois et al., *NPJ Vaccines*, 2020

- Overview of Sanofi Pasteur's adventitious virus detection by HTS test
- Panel of model viruses
- Validation
- Evolving Regulation
- Other Applications

Marius et al., *Biologicals* 2020, 67:49

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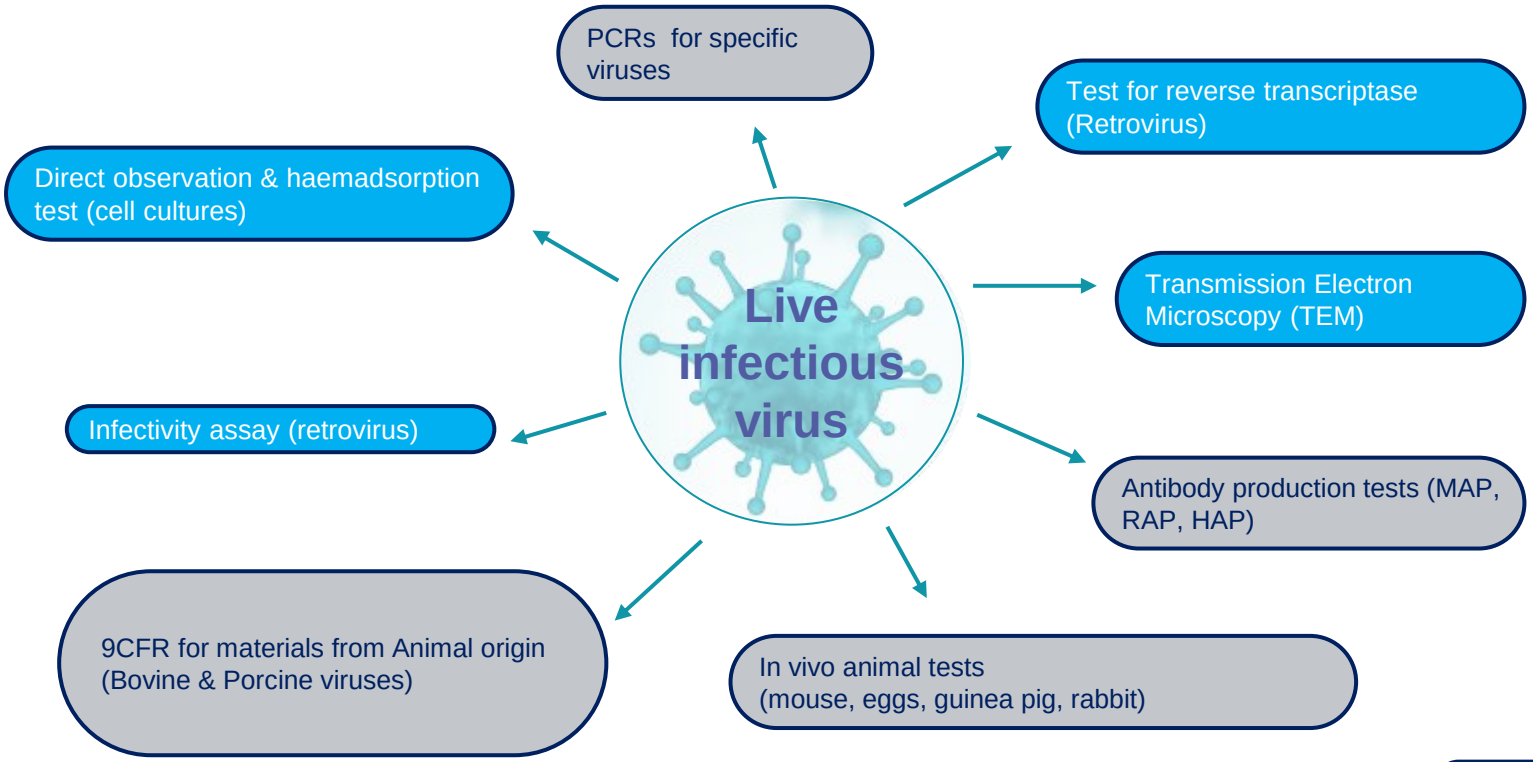
Viral Safety Tests



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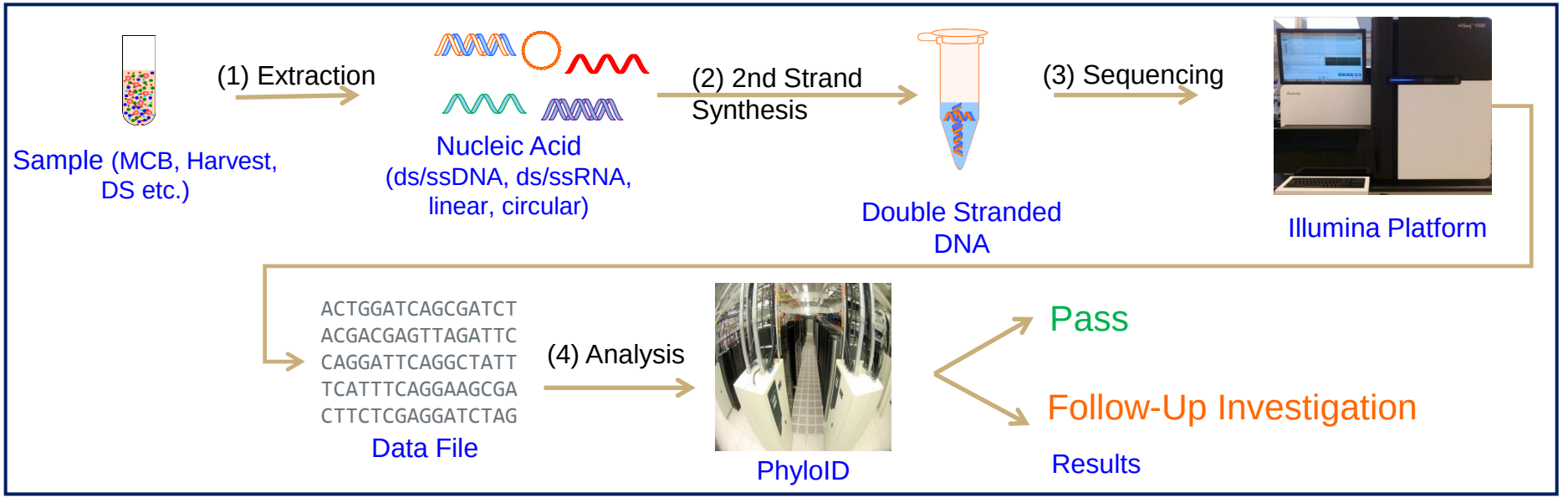
Potentially replaced by HTS

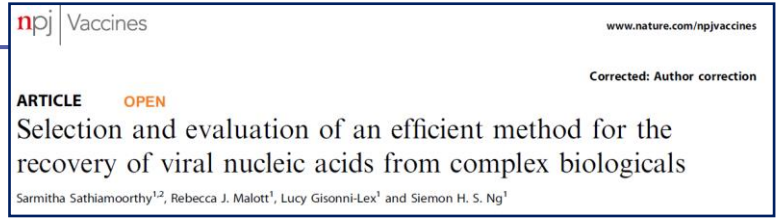
Overview of Sanofi Pasteur's Adventitious Virus Test

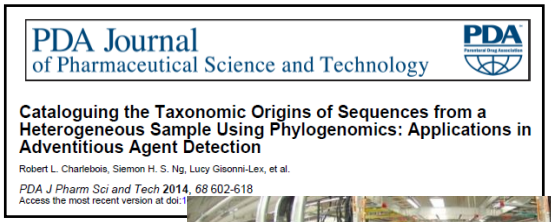
- **Designed to test any type of biological material (cell banks, viral seeds, crude harvests, drug substances, drug products)**
- **A whole-genome approach that takes advantage of very deep sequencing**



Method Development

- **Sample Extraction**
  - Compared between different extraction kits/methods using model viruses
- **Deep Sequencing**
- **In-house Bioinformatics**
  - PhyloID™- an automated analysis pipeline specifically designed for analyzing large sequence datasets for adventitious agent detection





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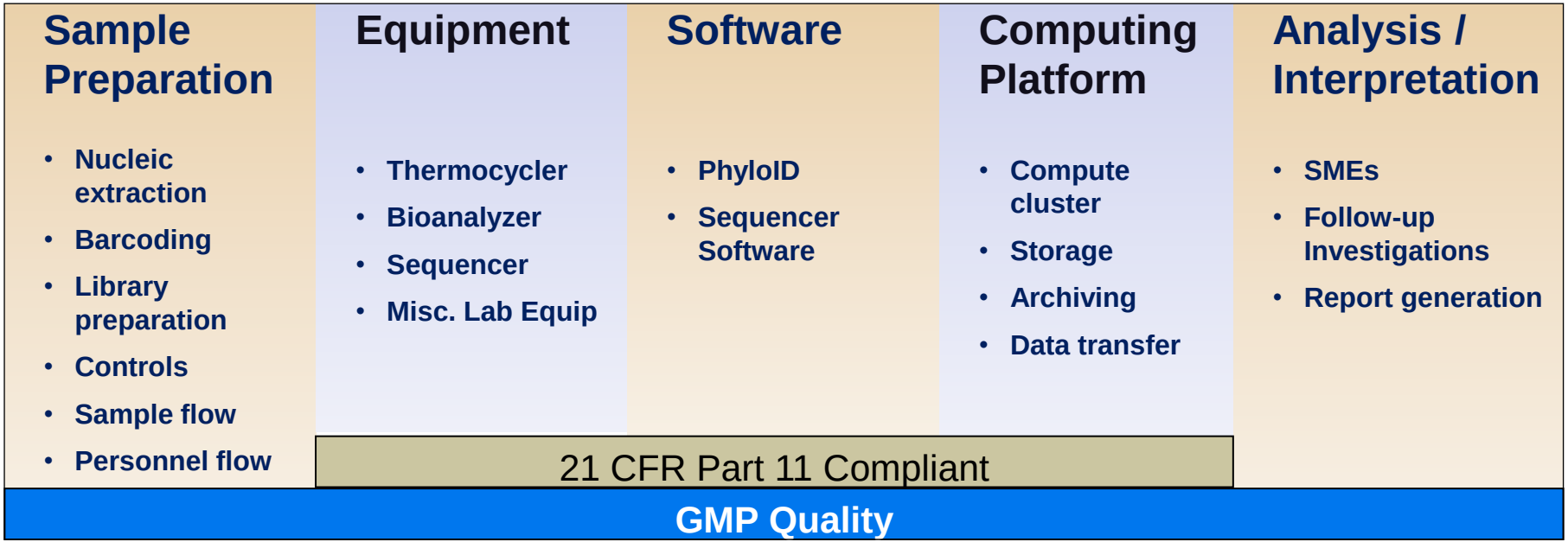
Controls

- **Minimize potential contaminations from the environment**
- **Assess sample extraction and library preparing**
- **Check points to ensure performance of the method**
- **Remove low quality sequencing data**

Sanofi Pasteur's Validation Approach



Model Viruses

- **Model viruses are critical to assessing the performance of an HTS adventitious virus detection test**
- **NIH published a study in 2014 (Gombold *et al.*,) that linked *in vivo* and *in vitro* tests for adventitious virus detection and demonstrated that the *in vivo* tests are not as sensitive**
  - 16 viruses across 9 viral families, representative of potential contaminants that could be introduced during vaccine production
  - Includes human and animal viruses from a variety of families, both RNA and DNA genomes as well as enveloped and non-enveloped viruses
- **This panel can be used to link HTS data to the published *in vivo and in vitro* data**

- Adenovirus 5
- Adenovirus 41
- Simian CMV
- HSV 1 (MacIntyre)
- Simian Virus 40
- BVDV (NY-1)
- Influenza A (A/PR/8/34)
- Measles virus (Edmonston)
- Mumps virus (Enders)
- Bovine Parainfluenzavirus Type 3
- Coxsackievirus A16
- Coxsackievirus B3
- Echovirus 11 (Gregory)
- Rhinovirus 2
- Vesicular Stomatitis Virus (Indiana)
- Rubella virus (M-33)

Summary of NIH study

Vaccine 32 (2014) 2916–2926

Contents lists available at ScienceDirect

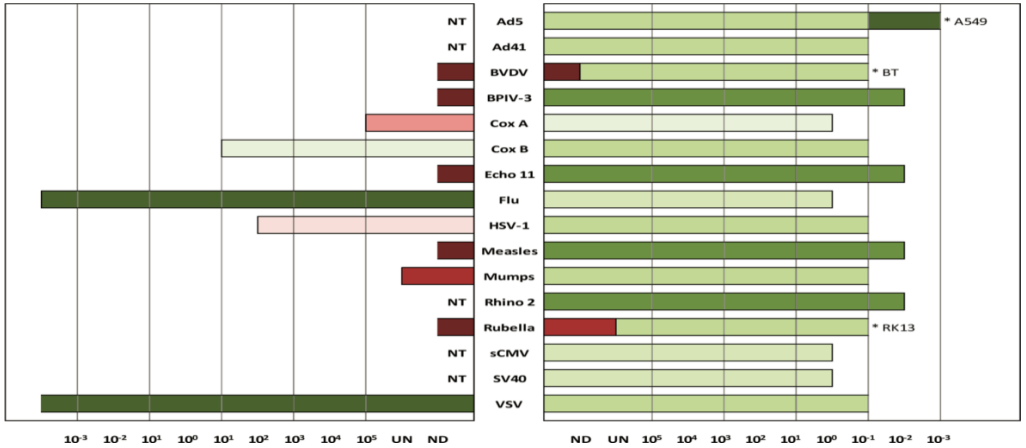
Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Systematic evaluation of *in vitro* and *in vivo* adventitious virus assays for the detection of viral contamination of cell banks and biological products☆

James Gombold[a], Stephen Karakasidis[a], Paula Niksa[b], John Podczasy[a], Kitti Neumann[a], James Richardson[c], Nandini Sane[c], Renita Johnson-Leva[c], Valerie Randolph[d], Jerald Sadoff[e], Phillip Minor[f], Alexander Schmidt[g], Paul Duncan[h], Rebecca L. Sheets[i,*]

In vivo



In vitro

Viral Family	Virus	Strain	Enveloped	Viral Genome	Genome Size (Kb)	Production Cell Line	Cell Line For Titration Assay
Adenoviridae	Adenovirus 5	Adenoid 75	No	dsDNA	36	A549	A549
	Adenovirus 41	N/A	No	dsDNA	34	HEK 293	HEK 293
Flaviviridae	Bovine Viral Diarrhea Virus	NY-1	Yes	ssRNA (+ve)	12.4	BT	BT
Herpesviridae	Herpes Simplex Virus Type 1	MacIntyre	Yes	dsDNA	150	Vero	Vero
	Simian Cytomegalovirus	CS6	Yes	dsDNA	221	MRC-5	MRC-5
Orthomyxoviridae	Influenza A	A/PR/8/34 (H1N1)	Yes	8 ssRNA (-ve)	12.5	MDCK	MDCK
Paramyxoviridae	Mumps	Enders	Yes	ssRNA (-ve)	15.4	Vero	Vero
	Bovine Parainfluenza Type 3	N/A	Yes	ssRNA (-ve)	15.5	Vero	Vero
	Measles	Edmonston	Yes	ssRNA (-ve)	15.9	Vero	Vero
Picornaviridae	Coxsackie A16	N/A	No	ssRNA (+ve)	7.4	Vero	Vero
	Coxsackie B3	N/A	No	ssRNA (+ve)	7.4	LLC-MK2	LLC-MK2
	Echovirus 11	Gregory	No	ssRNA (+ve)	7.4	LLC-MK2	LLC-MK2
	Rhinovirus 2	HGP	No	ssRNA (+ve)	7.1	HeLa	HeLa
Polyomaviridae	Simian Virus 40	Pa-57	No	dsDNA	5.2	Vero	Vero
Rhabdoviridae	Vesicular Stomatitis Virus	Indiana	Yes	ssRNA (-ve)	11.2	Vero	Vero
Togaviridae	Rubella	M-33	Yes	ssRNA (+ve)	9.7	BSC-1	RK-13

Equivalent Viral Stocks

- **Produced a set of equivalent viral stocks**
  - **Viruses were provided by the National Institute of Allergy and Infectious Diseases (via Rebecca Sheets)**
  - Followed the NIH protocols for virus propagation and titration (as close as possible)
    - Same-sourced cells, media formulations, multiplicity of infection (MOI), infection time, and harvest conditions
    - For some viruses, MOI, infection time and harvest conditions were modified
  - Determined the titer and genome copies for both SP stocks and the NIH stocks

- **Used this panel to assess the performance of HTS for adventitious virus detection**

Comparison of Sensitivity between HTS and *In Vivo* Tests

★ ***In vivo* test show better sensitivity than HTS (and *in vitro* test)**

★ **Equivalent between *in vivo* and HTS tests**

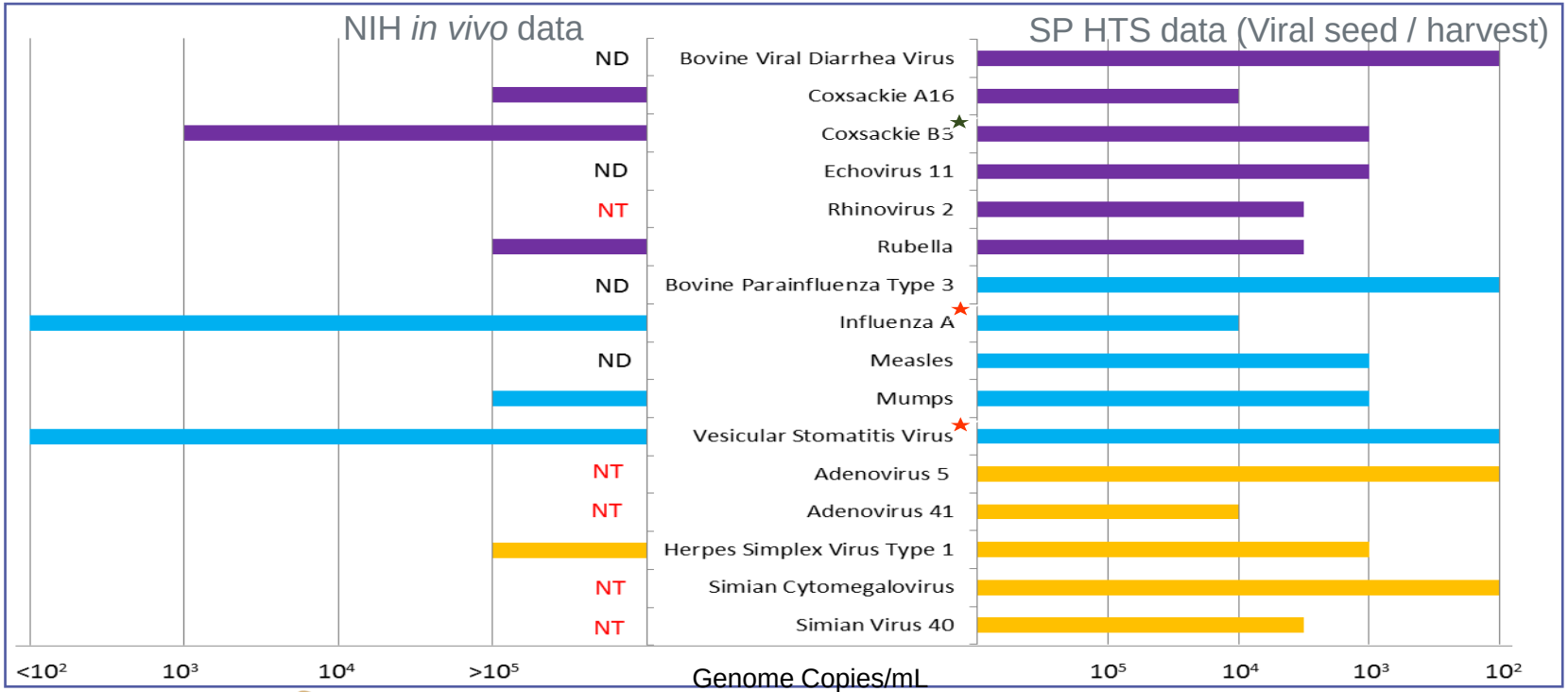
NT = not tested; ND = not detected;

Viral genome types: dsDNA; ssRNA (–ve); ssRNA (+ve)

npj | Vaccines
www.nature.com/npjvaccines
ARTICLE OPEN
Check for updates

Sensitivity and breadth of detection of high-throughput sequencing for adventitious virus detection

Robert L. Charlebois[1], Sarmitha Sathiamoorthy[2], Carine Logvinoff[3], Lucy Gisonni-Lex[3], Laurent Mallet[3] and Siemon H. S. Ng[3]



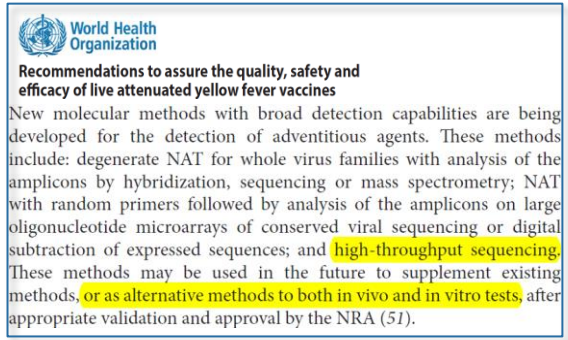
Method Validation

- **Validation as a limit test**
  - Not considered as a quantitative analysis
  - Controls for extractions and recovery for viral nucleic acids
- **Spiked in model viruses**
  - 16 NIH viruses spiked in at 10^4 genome copies into 1 mL of “matrix”
  - n=2
- **Specificity**
  - Demonstrated by a negative control extracted and sequenced in parallel
- **Breadth of detection confirmed**

Viruses Spiked into the Sample Matrix	Limit of Detection Performance Target (at 1×10^4 genome copies per mL)	Specificity Performance Target	Specificity Performance Target for Negative control
Adenovirus 5	+	+	-
Adenovirus 41	+	+	-
Bovine Viral Diarrhea Virus	+	+	-
Herpes Simplex Virus Type 1	+	+	-
Simian Cytomegalovirus	+	+	-
Influenza A	+	+	-
Mumps	+	+	-
Bovine Parainfluenza Type 3	+	+	-
Measles	+	+	-
Coxsackie A16	+	+	-
Coxsackie B3	+	+	-
Echovirus 11	+	+	-
Rhinovirus 2	+	+	-
Simian Virus 40	+	+	-
Vesicular Stomatitis Virus	+	+	-
Rubella	+	+	-
DNA Extraction Control	+	+	+
RNA Extraction Control	+	+	+

Regulatory Environment Evolution

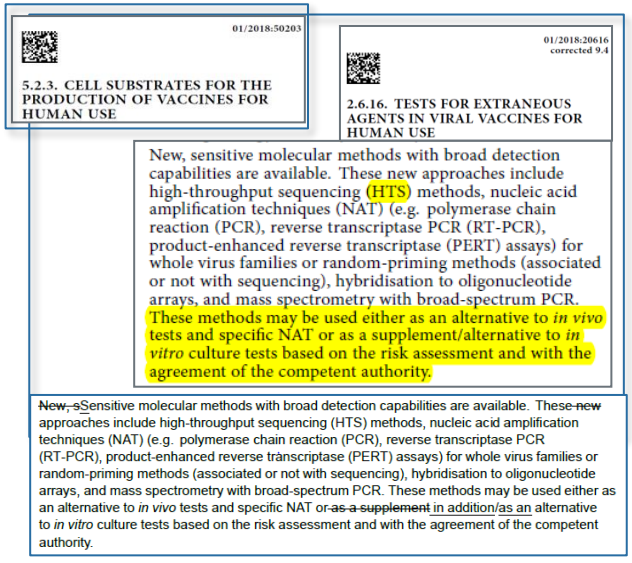
- **Evolution of WHO recommendations**
  - Cell substrates, Yellow Fever vaccine, Dengue Vaccine, IPV, …
- **Evolution of European Pharmacopoeia**
  - Ph. Eur. Chapter 5.2.14: "Substitution of *in vivo* method(s) by *in vitro* method(s) for the quality control of vaccines", version 9.3 published in July 2017, creation
  - Ph. Eur. Chapter 5.2.3: "Cell Substrates for the production of vaccines for human use", version 9:0 and updated version 9.3 in July 2017, revision
  - Ph. Eur. Chapter 2.6.16: "Tests for extraneous agents in viral vaccines for human use", version 9.3 published in July 2017
    - ↳under revision
    - ↳*in vivo* tests for adventitious agents only if it provides a risk mitigation evidenced by the Viral Risk Assessment



World Health Organization

Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines

New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include: degenerate NAT for whole virus families with analysis of the amplicons by hybridization, sequencing or mass spectrometry; NAT with random primers followed by analysis of the amplicons on large oligonucleotide microarrays of conserved viral sequencing or digital subtraction of expressed sequences; and high-throughput sequencing. These methods may be used in the future to supplement existing methods, or as alternative methods to both in vivo and in vitro tests, after appropriate validation and approval by the NRA (*51*).



01/2018:50203

5.2.3. CELL SUBSTRATES FOR THE PRODUCTION OF VACCINES FOR HUMAN USE

01/2018:20616 corrected 9.4

2.6.16. TESTS FOR EXTRANEOUS AGENTS IN VIRAL VACCINES FOR HUMAN USE

New, sensitive molecular methods with broad detection capabilities are available. These new approaches include high-throughput sequencing (HTS) methods, nucleic acid amplification techniques (NAT) (e.g. polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), product-enhanced reverse transcriptase (PERT) assays) for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays, and mass spectrometry with broad-spectrum PCR. These methods may be used either as an alternative to *in vivo* tests and specific NAT or as a supplement/alternative to *in vitro* culture tests based on the risk assessment and with the agreement of the competent authority.

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Other HTS Applications

Variant Analysis by HTS

Journal of Virological Methods 246 (2017) 75–80

Contents lists available at ScienceDirect

Journal of Virological Methods

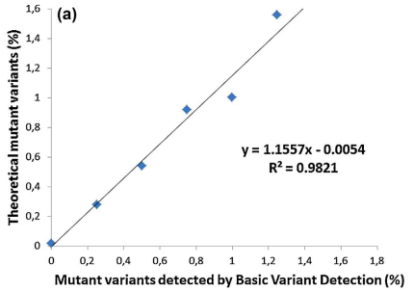
journal homepage: www.elsevier.com/locate/jviromet

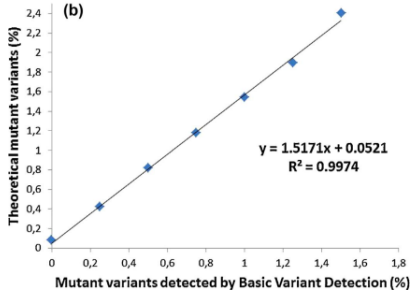
Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing

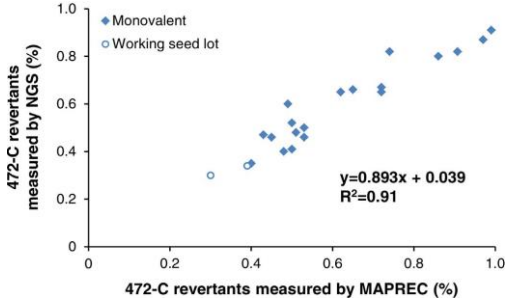
Eric Sarcey[a,*], Aurélie Serres[a], Fabrice Tindy[a], Audrey Chareyre[a], Siemon Ng[b], Marine Nicolas[a], Emmanuelle Vetter[a], Thierry Bonnevay[a], Eric Abachin[a], Laurent Mallet[a]

[a] Sanofi Pasteur, Analytical Research and Development Department EU, Campus Mérieux—1541, Avenue Marcel Mérieux, 69280, Marcy L'Etoile, France
[b] Sanofi Pasteur, Microbiology & Virology Platform, Department of Analytical Research & Development North America, 1755 Steeles Avenue West, Toronto, Ontario M2R 3T4, Canada

- **Mutant analysis by polymerase chain reaction (PCR) and restriction enzyme cleavage (MAPREC) is used to monitor the presence of a specific nucleotide variant that's correlated to neurovirulent revertants**
  - Labor intensive and requires use of radioactive labelling for sensitivity
- **Demonstrated a high correlations between MAPREC and HTS which supports the replacement of MAPREC**
  - Assessment of variants by HTS can differentiate frequencies as low as 0.1%







(a) Direct HTS without PCR [Synthetic]
(b) HTS after PCR [Synthetic]
(c) Compared against historical results

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Genetic Stability / Identity Testing

- **Confirm that seeds or cells banks are genetically stable over the entire manufacturing process**
- **Positive identification of engineered mutations**
- **HTS is more sensitive than traditional Sanger sequencing**
  - Assess specific regions or the entire genome
  - Sequence with no need for designing primers
- **Implemented and validated an in-house bioinformatics with features not available in commercial software:**
  - Find statistically significant differences between HTS data sets
  - Compare the consensus sequence from 1 or two HTS data set against a reference sequence
  - Ensure GMP compliance (User access control, audit trail, storage)

The Future

- **Accelerating Analytics**
  - Demonstrated that HTS can be fully validated an HTS adventitious virus detection assay and with better sensitivity than the *in vivo* tests
  - HTS can also be applied to variant detection and genetic stability analysis
- **Regulatory harmonization**
  - Recent regulation updates allows for streamlining of the testing package with the replacement of the *in vivo* adventitious virus tests by an HTS adventitious virus detection test method
- **Post-Covid**
  - Novel manufacturing platforms

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