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Session V: Getting Vaccines to the Market: Case studies

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Time to Apply the 3 R's to Virus Testing?

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Disclaimer

- This presentation represents solely the opinions of the speaker and does not reflect any U.S. government policies or the opinions of the National Institutes of Health or the Food & Drug Administration.

Context

- 3 R's – reduce, refine, or replace the use of animals in product testing
  - Lack of regulatory convergence
- EU has directive (mandatory law) requiring that 3 R's be applied; whenever *in vitro* methods are available, *in vivo* tests should not be used.
  - Illegal for European companies to not comply, but if they sell to US or other countries that still require *in vivo* tests, they are in catch 22.
  - EDQM is changing EP in consideration of data from study I will present
- US has public law to REVIEW their regulations to see where 3 R's could be applied, but only policy to actually apply 3 R's
  - Despite data I will present, FDA has not changed requirements (policy is not translated into action)

Introduction

- **Routine Tests for Adventitious Viruses**
  - ***in vivo* with mortality or morbidity read-outs**
  - **In tissue culture with CPE & HAd read-outs**
  - **Transmission Electron Microscopy**
  - **Specific PCRs for selected viruses**
  - **PCR-based reverse transcriptase assay**
  - **(infectivity for retroviruses)**
  - **Bovine, porcine viruses (9 CFR tests in cell culture with CPE, HAd, and IFA read-outs)**
  - **(MAP, RAP, HAP – *in vivo* or PCR)**

“Routine” Adventitious Virus Tests Purpose of Work

- Provide regulators and manufacturers with information needed for decision-making
  - Such info normally comes from assay validation
- Provide baseline data to serve as basis of comparison for new methods
- Provide protocols and viral stocks to permit “direct” comparisons by developers of new methods
- Determine “value added” by *in vivo* methods in consideration of 3 R’s policy

“Routine” Adventitious Virus Tests Breadth & Sensitivity

- These tests were developed for clinical diagnostics in mid-20th century
- Initially used to detect SPECIFIC adventitious agents
- Use expanded to broad general screening assays
- Breadth/sensitivity had not been systematically assessed & published
- Not validated in the manner currently developed assays would be required
- No regulatory requirements to do so and costly to do

“Routine” Adventitious Virus Tests Implementation Phase

- The prime contractor, Advanced BioScience Laboratories, awarded task to Charles River Labs to implement this project
  - Compliant with Good Laboratory/Manufacturing Practices
  - Experienced with routine adventitious agent testing
  - *In vivo* and *in vitro* capabilities
  - Virology expertise to prepare and characterize viral stocks required

“Routine” Adventitious Virus Tests Implementation Phase (2)

- Viral stocks prepared in cell culture
  - Titrated in production cell line or positive control cell line
  - Characterized for purity & identity
- *In vivo* testing
  - Test at highest concentration for breadth
  - If positive, sensitivity determined by titration (dilutions)
- *In vitro* testing
  - Breadth and sensitivity assessed simultaneously

“Routine” Adventitious Virus Tests Results and Conclusions

The results can answer questions such as:

- **Is using two human cell lines useful?**
- **Is a 14-day *in vitro* test sufficient or are 28 days needed?**
- **Is sub-passage useful for suckling mouse test sensitivity?**
- **Which is more sensitive – *in vitro* or *in vivo*?**

- **Yes, MRC-5 & HeLa had different sensitivities, sometimes one was better, sometimes the other**
- **28 days more sensitive in some cases**
- **No, for the viruses tested**
- **With the exception of flu & VSV, the *in vitro* tests were always more sensitive, generally by logs, sometimes the difference between detecting & not detecting**

“Routine” Adventitious Virus Tests Results and Conclusion

Table 4. *In Vitro* Limit of Detection of Research Virus Stocks

Virus	Vero		MRC-5		HeLa		Other	
	CPE	HA/HAD/IF[a]	CPE	HA/HAD/IF[a]	CPE	HA/HAD/IF[a]	CPE	HA/HAD/IF[a]
Adenovirus 5								
Adenovirus 41								
BVDV								
BoPIV-3							nd [b]	nd
Coxsackie A16								
Coxsackie B3						nd	nd	nd
Echovirus 11							nd	nd
Influenza A							nd	nd
HSV-1							nd	nd
Measles							nd	nd
Mumps							nd	nd
Rhinovirus 2		nd		nd		nd	nd	nd
Rubella								
Simian CMV								
SV-40							nd	nd
VSV		nd		nd		nd	nd	nd

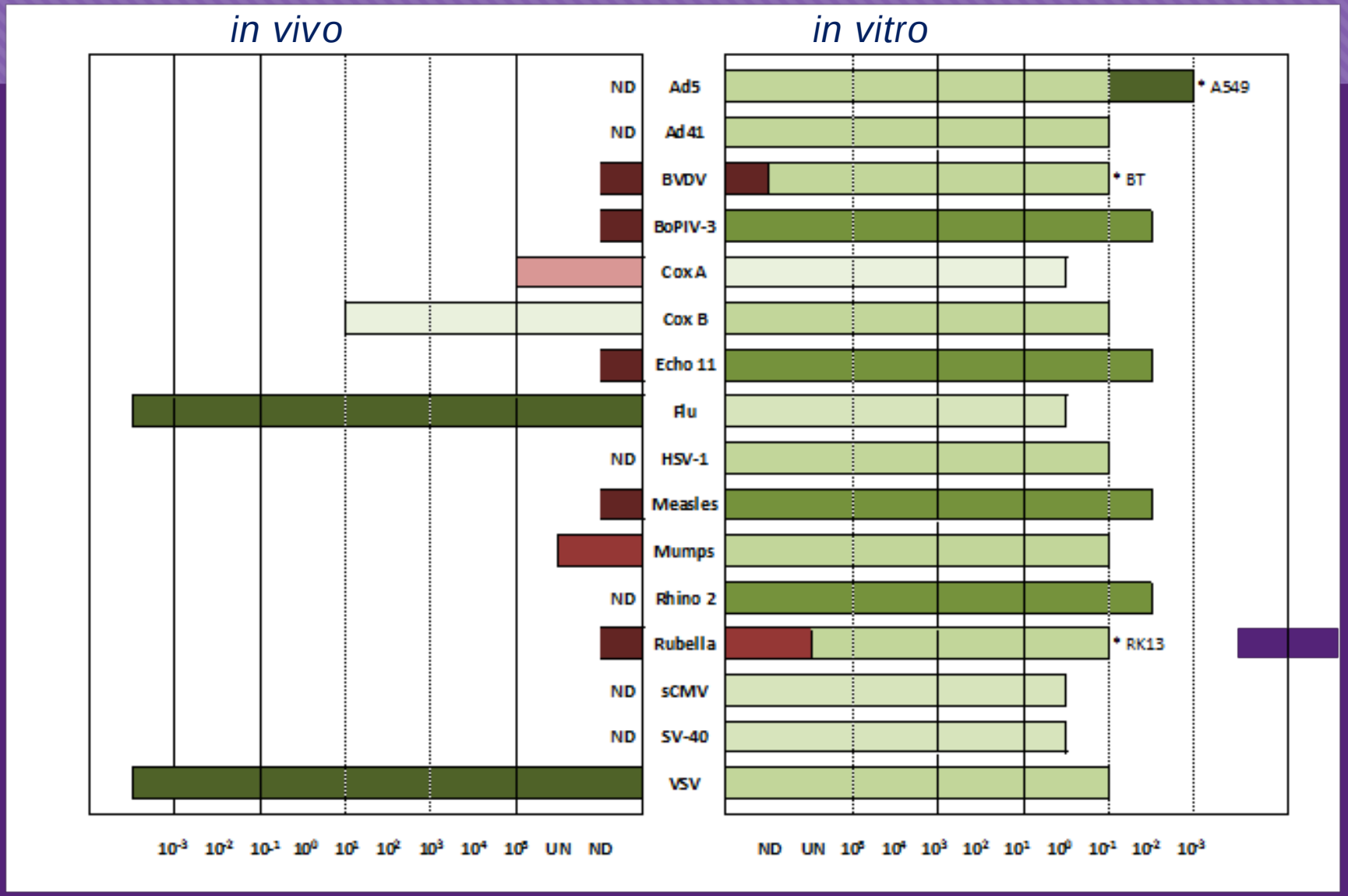
0.001 ID	
0.01 ID	
0.1 ID	
1.0 ID	
10.0 ID	
100.0 ID	
1,000.0 ID	
100,000.0 ID	
Undiluted	
Undetected	

[a]All virus-infected cultures were tested for hemadsorption activity except BVDV (immunofluorescence), influenza A and rubella (hemagglutination) and rhinovirus 2 and VSV (CPE only)

[b]Not done

[b] Detection by immunofluorescence

“Routine” Adventitious Virus Tests Results



“Routine” Adventitious Virus Tests Outcomes/Deliverables

- Viral stocks will be made available through the NIAID/DAIDS *Reagent Resource Support Program for AIDS Vaccine Development*
  - http://www.niaid.nih.gov/topics/hivaids/research/vaccines/resources/reagent/pages/default.aspx
  - A research repository, not a regulatory authority control lab reagent repository
  - Not international reference materials, but research reagents
- Protocols for virus preparation, titration, and for *in vivo* and *in vitro* test methods will also be made available

“Routine” Adventitious Virus Tests Acknowledgements

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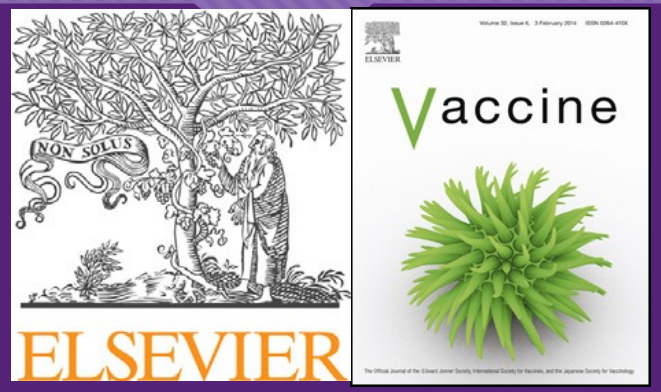
Publication



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

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Time to Apply 3 R's to Virus Testing?

YES