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Systematically Characterizing Adventitious Agent Tests for Biologics

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ECI Vaccine Technology IV
Albufiera, Portugal

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

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Outline

- Background and Context of Projects
- Bovine and Porcine Viruses
  - Scope and Purpose of Work
  - Methods
  - Results and Conclusions
- “Routine” adventitious virus tests
  - Scope and Purpose of Work
  - Planning
  - Implementation
  - Results and Conclusions
  - Future Considerations
- Summary

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Background and Context

- **DAIDS and IABS co-sponsored Vaccine Cell Substrates 2004**
  - **3 day conference, 6 themes**
  - **2 of those themes were Bovine (& porcine) Viruses in raw materials, Viral Adventitious Agent Test Methods**
  - **One purpose of conference was to identify research gaps that preclude decision-making, since DAIDS is a funding organization that could potentially fund gap-filling research**
  - **Progress on Cell Substrate policy was required to facilitate the development of HIV/AIDS vaccine candidates (novel approaches, novel vectors, novel cell substrates)**

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Background and Context (2)

- Panel discussions led to recommendations
- Bovine (and porcine) viruses
  - A systematic consideration should be given to the agents listed in 9 CFR with regard to relevance to use of animal-derived raw materials in production of human biologicals
- Viral Adventitious Agent Test Methods
  - Sensitivity and breadth of existing tests are presumed from historical experience and should be evaluated systematically

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Bovine and Porcine Viruses
Scope and Purpose of Work

- **Review literature**
  - **To determine whether the bovine viruses specified in 9 CFR 113.47 are capable of infecting humans or display human host range (e.g., by infecting human cells in culture or producing antibodies in natural- or laboratory-exposed humans)**
  - **To determine if there are other bovine viruses of concern (displaying bovine and human host range) that could be predicted to be detected by the 9 CFR procedures (including CPE and HAd/HAg on indicator cells used in test)**

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Bovine and Porcine Viruses
Scope and Purpose of Work (2)

- Review literature
  - To identify porcine viruses (in addition to porcine parvovirus) having human host range and could contaminate porcine trypsin
  - To predict whether these porcine viruses would be detected by the 9 CFR test methods
- Strictly a literature review and predictions would need to be verified in the laboratory – outside scope of project
- Included more than traditionally zoonotic viruses because biologicals are administered by routes that would bypass normal host defenses to natural infections

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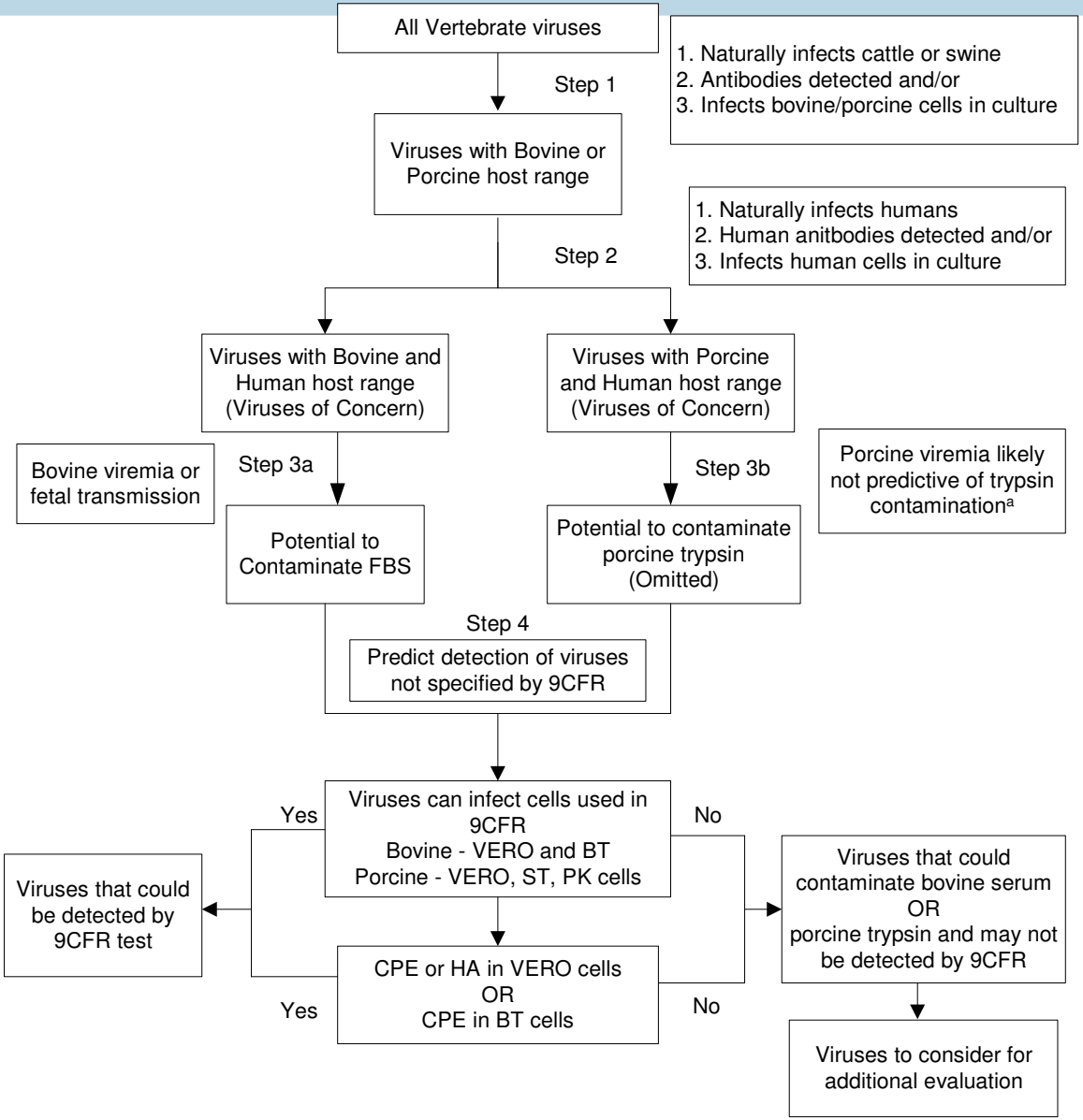
Bovine and Porcine Viruses Methods



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Bovine and Porcine Viruses
Results and Conclusion

- Some bovine viruses in the 9 CFR test are probably not important to human biologicals, but many more bovine and porcine viruses than are predicted to be detectable by the 9 CFR tests may be of concern
- Even within virus families, one or more members may be detected, but others may not
- Even within a virus type (e.g., reoviruses), it isn't clear that all strains would be detected (e.g., if Reo-3 is detected by the specific antisera used for IFA, what is sensitivity to detect Reo-1 and -2?)

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Bovine and Porcine Viruses Results and Conclusions (2)

Virus family	Bovine				Porcine		
	Viruses with HHR	Capable of fetal transmission	May contaminate FBS	Predicted to cause CPE or HAd in 9CFR	Viruses with HHR	Predicted to cause CPE or HAd in 9CFR	IFA read out in 9CFR testing
Adenoviridae	■	■	■	■			■
Anelloviridae [proposed family name]	■	■	■		■		
Bornaviridae	■	■	■				
Bunyaviridae	■	■	■		■		
Caliciviridae	■	■	■	■	■	■	
Circoviridae	■	■	■		■	■	
Coronaviridae	■		■	■	■		
Filoviridae					■	■	
Flaviviridae	■	■	■	■	■	■	■
Hepeviridae [proposed family name]	■	Unknown	■		■		
Herpesviridae	■	■	■	■	■	■	
Orthomyxoviridae	■		■	■	■	■	
Papillomaviridae	■	Likely	■				
Paramyxoviridae	■	■	■	■	■	■	■
Parvoviridae	■	■	■		■	■	■
Picornaviridae	■	■	■	■	■	■	
Polyomaviridae	■	■	■				
Poxviridae	■	■	■	■			
Reoviridae	■	■	■	■	■	■	■
Retroviridae	■	■	■	■	■		
Rhabdoviridae	■	■	■	■	■	■	■
Togaviridae	■	Unknown	■	■	■	■	

■ At least one member of the family has the attribute

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Bovine and Porcine Viruses Results and Conclusion (3)

- Recommendations (alternatives)
  - No change
  - Modify 9 CFR tests
  - Require testing of fetal bovine sera for anti-viral antibodies
  - Require gamma irradiation of bovine sera, use recombinant trypsin, use serum-free or animal-derived materials-free media
  - Consider incorporating the minipool concept
  - Consider additional tests for specific viruses
  - Incorporate virus-family testing
  - Consider using new test methods
  - And more

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Bovine and Porcine Viruses
Find out more

This study is published in *Biologicals* 39(6)359-69 Nov. 2011

"Evaluation Of The Human Host Range Of Bovine And Porcine Viruses That May Contaminate Bovine Serum And Porcine Trypsin Used In The Manufacture Of Biological Products"

Carol Marcus-Sekura, James Richardson, Nandini Sane, Rebecca Harston, Rebecca Sheets

- **CMS – BASI (sub-contract)**
- **JR, NS, RH – Advanced BioScience Laboratory (prime contractor)**
- **Also acknowledge: Jack Hill, HMJF/DAIDS, Renita Johnson-Leva, ABL**

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"Routine" Adventitious Virus Tests Scope of Work

- **Systematically characterize the breadth & sensitivity of the "routine" adventitious virus tests**
  - ***In vivo*** **(so-called "Inapparent Viruses" Test)**
  - ***In vitro*** **(cell culture)**

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"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 NIH's 3 R's policy

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"Routine" Adventitious Virus Tests Planning Phase

- A panel of experts was convened
- Panel discussed
  - Project utility
  - Project questions
  - Design and methods
  - Practical considerations
    - Though not intended to support a regulatory filing, study should be conducted in accordance with Good Laboratory/Manufacturing Practices (GLP/GMP)
    - Take a matrix (checkerboard) approach
    - Selected choice of viruses
    - Viral stocks must be cultured and titered, recognizing potential bias this introduced into study design

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"Routine" Adventitious Virus Tests Methods

- *In Vitro* tests
  - monolayers of at least 3 cell types, look for CPE
  - tests for hemadsorption and hemagglutination or immunofluorescence at end of culture period
- *In Vivo* tests
  - adult and suckling mice
  - when appropriate
    - embryonated hens' eggs
    - guinea pigs
    - rabbits

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“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 has not been systematically assessed and published
- Not validated in the manner currently developed assays would be required
- No regulatory requirements to do so and costly to do

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"Routine" Adventitious Virus Tests Matrix (Checkerboard) approach

	MRC-5	Vero	HeLa	eggs	adult mice	suckling mice
mumps	X	X	X	X		
measles	X	X	X	X		
rubella	X	X	X	X		
Flu	X	X	X	X		
HSV-1		X			X	X
sCMV	X		X			
SV40		X				
Coxsackie A	X	X	X			X
Coxsackie B	X	X	X			X
echovirus	X	X	X			X
VSV		X		X	X	X
PIV-3		X		X		
BVDV		IFA			X	X
rhinovirus	X	X	X			
Ad41	X	X	X			
Ad5	X	X	X			

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"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

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"Routine" Adventitious Virus Tests Implementation Phase (2)

- Viral stocks prepared in cell culture
  - Titered
  - 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

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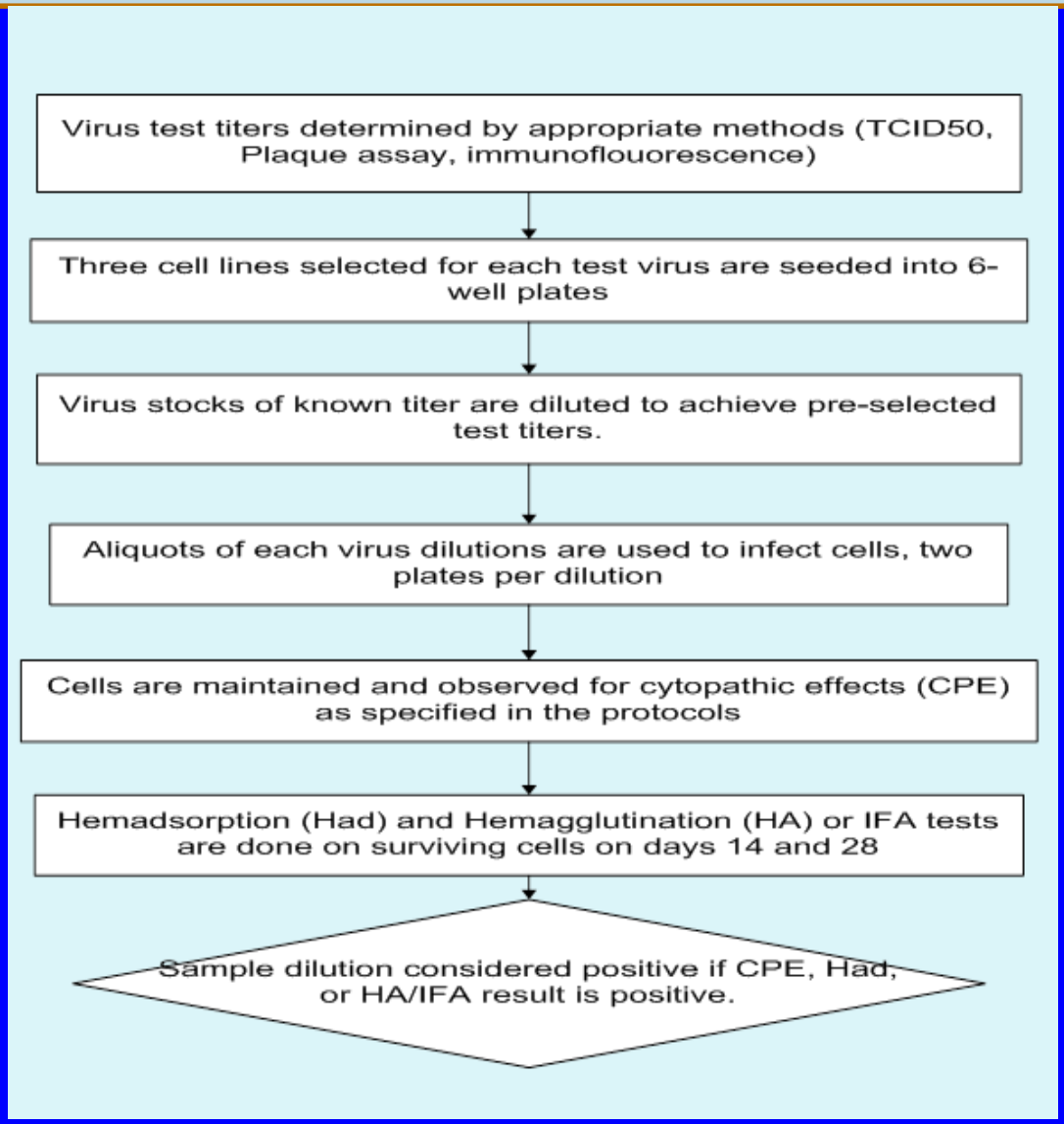
"Routine" Adventitious Virus Tests
In Vitro Adventitious Virus Assay



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"Routine" Adventitious Virus Tests
In Vivo Adventitious Virus Assay

- Study design driven by ethical considerations
- Current acceptance criteria of 80% survival rate was used
- LOD was defined as the virus titer resulting in ≤ 20% mortality (LD20)
- Initial study used undiluted stock virus
- Titration study determined LD20 for each virus sample
  - multiple inoculation groups of 10 mice and 10 eggs each received virus at various titers (determined by *in vitro* titration) to see which resulted only in ≤ 20% mortality
- To minimize cross-contamination
  - One virus per room at any given time
  - Performed in a Class II Biological Safety Cabinet
  - Animals were housed in isolators in filter-topped Microisolator® cages in a negative pressure work area (100% exhausted)

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"Routine" Adventitious Virus Tests Results and Conclusions

The results can answer questions such as:

- **Is using two human cell lines useful?**
  - **Yes, MRC-5 & HeLa had different sensitivities, sometimes one was better, sometimes the other**
- **Is a 14-day *in vitro* test sufficient or are 28 days needed?**
  - **28 days more sensitive in some cases**
- **Is sub-passage useful for suckling mouse test sensitivity?**
  - **No, for the viruses tested**
- **Which is more sensitive – *in vitro* or *in vivo*?**
  - **With the exception of flu, the *in vitro* tests were always more sensitive, generally by logs, sometimes the difference between detecting and not detecting**

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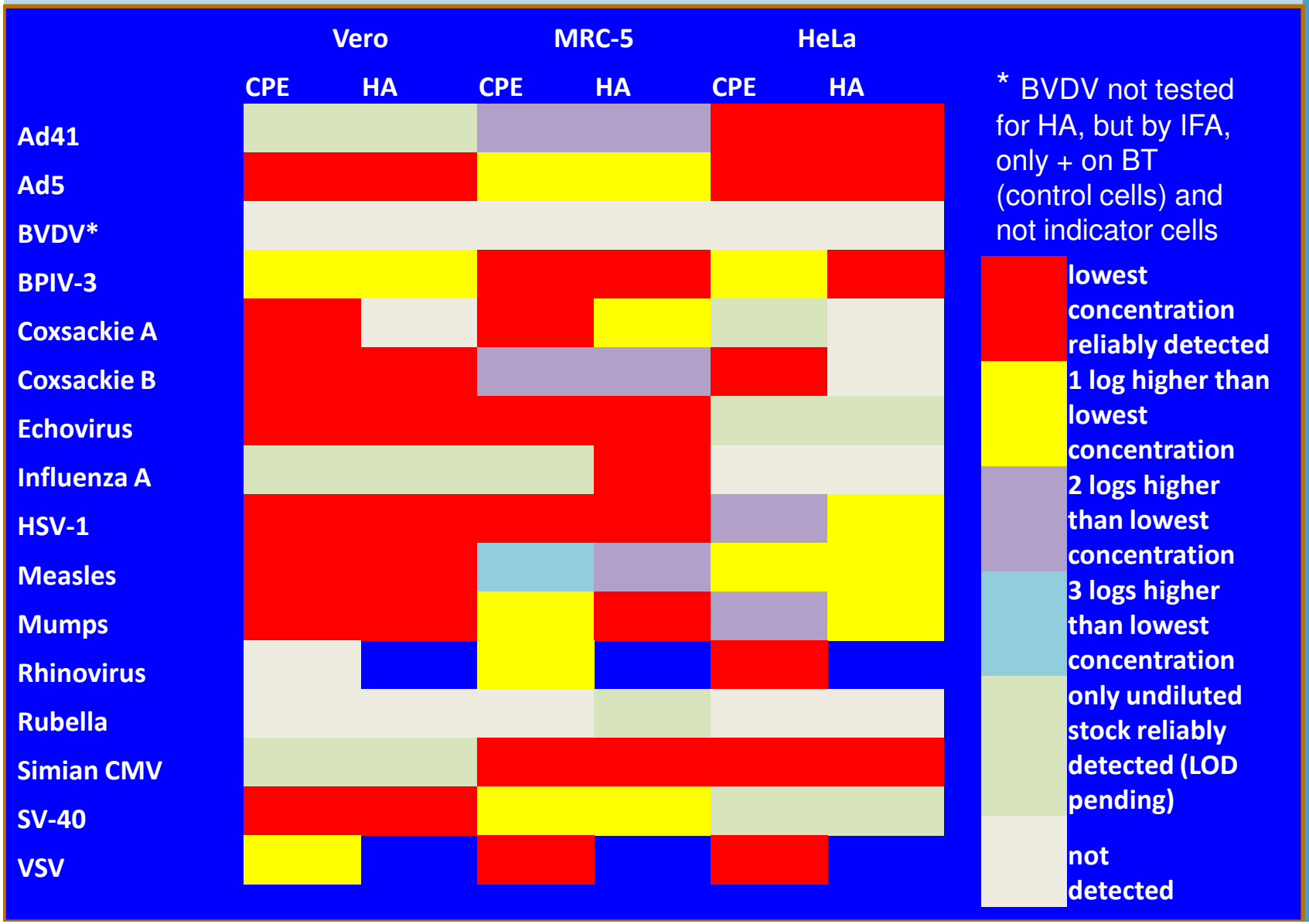
"Routine" Adventitious Virus Tests Results and Conclusion



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"Routine" Adventitious Virus Tests
In Vivo Adventitious Virus Assay (2)

Virus↓ Test→	Vero		MRC-5		HeLa		Suckling Mice		Eggs		
	CPE	HA	CPE	HA	CPE	HA	Original	Sub-passage	Allantoic		Yolk Sac
									Death	HA	Death
Coxsackie A $6.3x10^6$	10	ND	10	100	10^5	ND	ND	ND	nt	nt	nt
Measles $9.3x10^5$	0.01	0.01	10	1	0.1	0.1	nt	nt	ND	ND	ND
Echovirus $2x10^7$	0.01	0.01	0.01	0.01	100	UD	ND	ND	nt	nt	nt
Influenza A $6.3x10^7$	UD	UD	UD	1	ND	ND	nt	nt	0.1	0.01	0.001

LOD or LD20 values given
ND – not detected, negative at highest concentration
UD – Undiluted nt – not tested

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"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

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"Routine" Adventitious Virus Tests Future Considerations

- Provide developers of new methods with viral stocks/protocols/baseline data
- Complete the missing cells of the matrix
- Expand the list of viruses to new viral families
- Replicate with multiple strains of a particular virus
- Test field isolates
  - Most relevant to bovine/porcine viruses and to products made in primary cell cultures/tissues/ animals
  - Culture-adapted strains relevant to cell culture-derived products, because contaminant would best amplify and contaminate product, if adapted to the production cell culture

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“Routine” Adventitious Virus Tests Acknowledgements

- Expert Panel
  - Phil Minor, NIBSC
  - Keith Peden, CBER
  - Alex Schmidt, NIAID
  - Becky Sheets, NIAID
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  - Valerie Randolph, Pfizer
  - Jerry Sadoff, Crucell
- ABL
  - James Richardson
  - Nandini Sane
  - Renita Johnson-Leva
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    - Jim Gombold
    - Stephen Karakasidis
    - John Podczasy
    - Michelle Walker
    - Barry Rosenblatt
    - Lana Mogilyanskaya
    - Sveta Sherbaty
  - IVB (Wilmington, MA)
    - Paula Niksa
    - William Shek

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Summary

- Two of several NIAID-funded projects on adventitious agent testing highlighted in this talk
  - Bovine and Porcine Viruses
    - Publication in *Biologicals* 39(6)359-69 Nov. 2011
  - "Routine" Adventitious Virus Tests
    - Will be published
    - Viral stocks will be available as research reagents
    - Protocols will also be available
- Additional activities not highlighted
  - CBER/OVRR 2010 Guidance on cell substrates
  - WHO 2010 Guidance on cell substrates
  - Inter-Agency Agreement with CBER
- Welcome opportunities to collaborate or continue progressing cell substrate, adventitious agent testing, and 3 R's policy issues

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Extra Slides

"Routine" Adventitious Virus Tests
Adult Mice Test Methods

- Originally performed for purpose of detecting LCMV or other viruses
- ≥20 adult mice
- i.p. with 0.5 mL, i.c., with 0.03 mL
- Mice must survive 21 days
  - ≥80% survival
  - No sign of viral infection
- Believed to be capable of detecting LCMV, coxsackieviruses, flaviviruses, rabies

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"Routine" Adventitious Virus Tests Suckling Mice Test Methods

- Originally performed for purpose of detecting Coxsackieviruses (particularly type A)
- ≥20 mice less than 24 hours old
- i.c., 0.01 mL, i.p., 0.1 mL
- 14 days
- Subinoculation into additional mice for 14 days
- Mice must survive
  - ≥80% survival both inoculations
  - No signs of viral infection
- Believed to be capable of detecting coxsackieviruses, other picornaviruses (polioviruses, echoviruses), alphaviruses, herpesviruses (HSV), flaviviruses, rabies, many murine agents, others

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"Routine" Adventitious Virus Tests Embryonated Hens' Eggs Test Methods

- 10-11 day-old embryos, 0.5 mL allantoic route, 3 days, HA
  - Believed to be capable of detecting orthomyxoviruses (influenzaviruses), paramyxoviruses (mumps, measles, parainfluenzaviruses), alphaviruses
- 6-7 day-old embryos, 0.5 mL yolk sac route, >9 days, survival
  - Believed to be capable of detecting herpesviruses (HSV), poxviruses, rhabdoviruses, rickettsiae, mycoplasmas, bacteria

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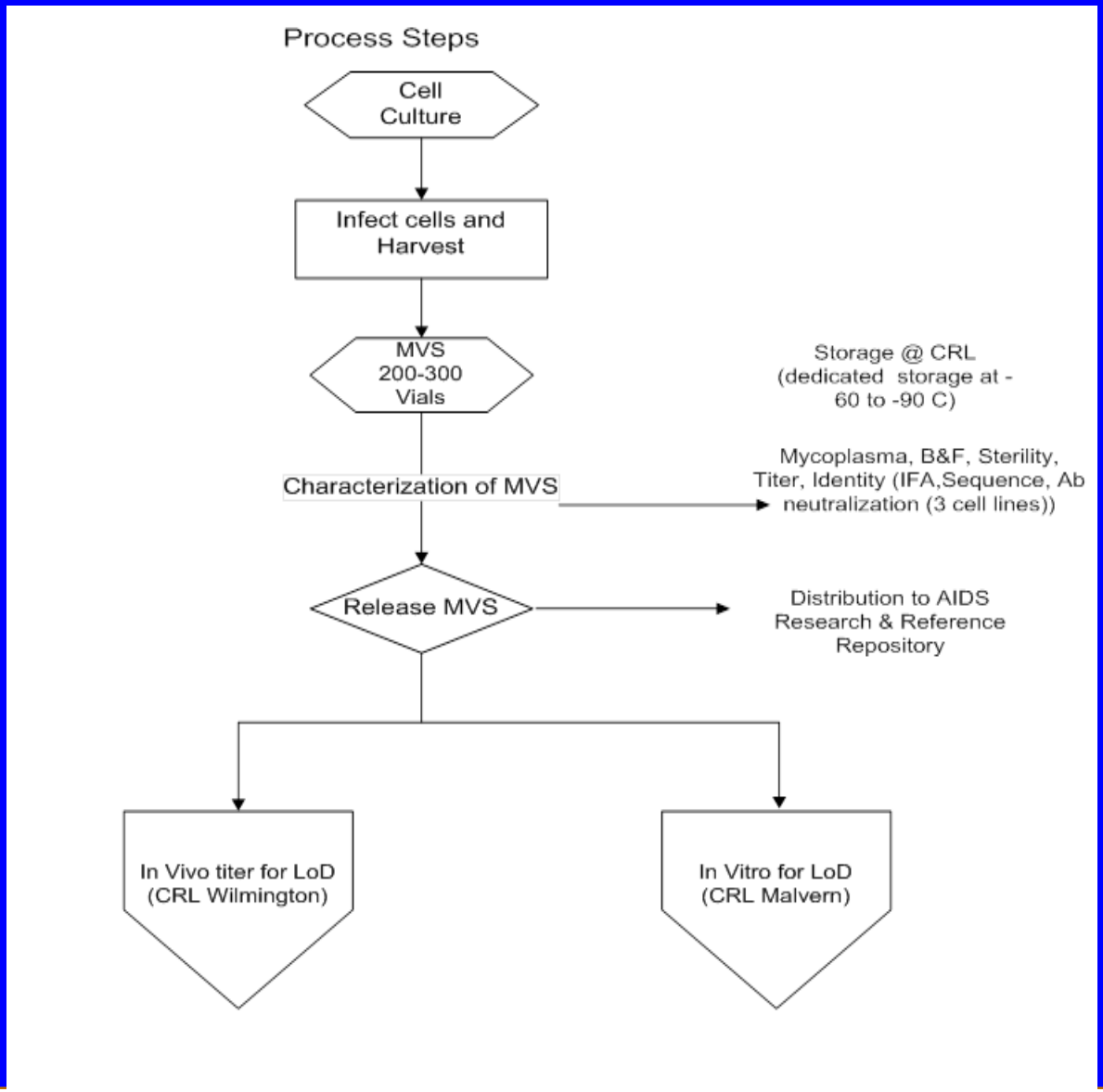
“Routine” Adventitious Virus Tests Preparation of Virus Stocks



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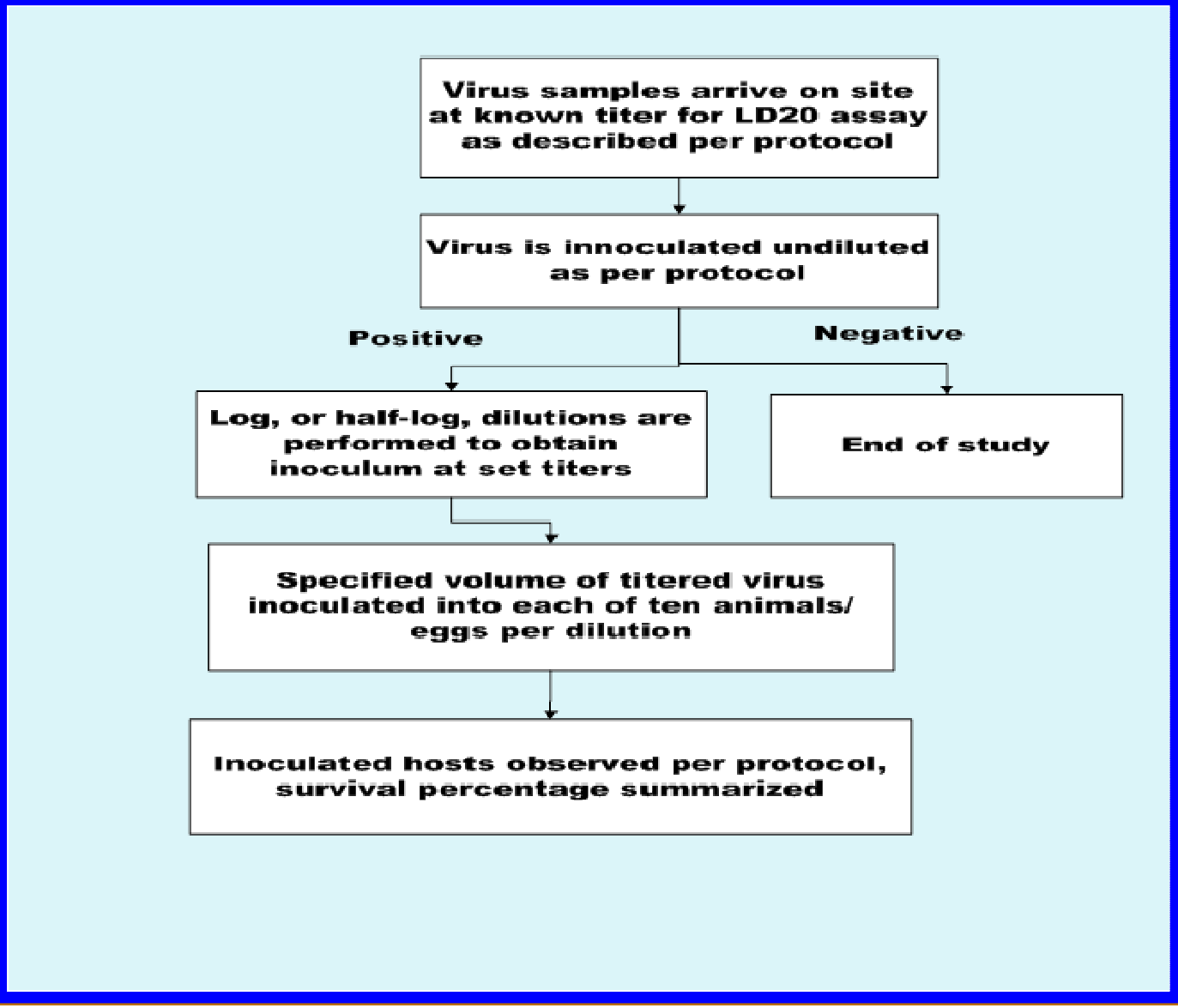
"Routine" Adventitious Virus Tests
In Vivo Adventitious Virus Assay (2)



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