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Process Scale-up and Optimizatio... for Production of High Efficacy Ora... Rabies Vaccine

Amine Kamen

VT III, Nuevo Vallarta, Mexico June 9, 2010

Conseil national de recherches Canada | National Research Council Canada

Canada

Outlines

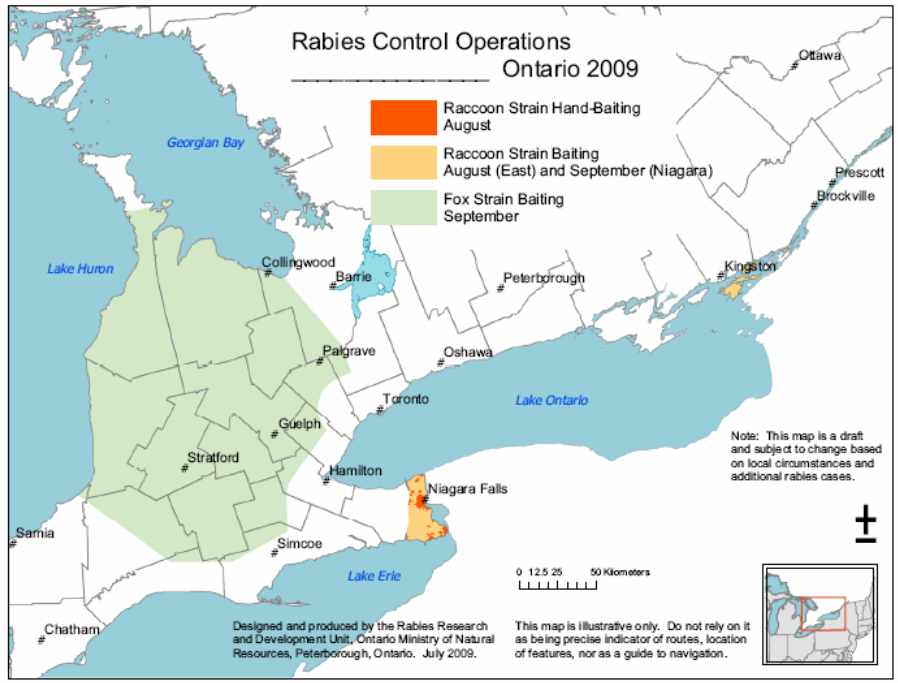
- Introduction
- Process development
- Field trials results
- Conclusions and Perspectives

Rabies: Old Disease New Challenges

- Rabies is an important causative agent of disease resulting in an acute infection of the nervous system and death
- Rabies remains endemic throughout the world.
  - 55,000 deaths per year (WHO)
  - Major Public health issue
- Global reservoir in domestic and wildlife animals including dogs, foxes, raccoons, skunks, coyotes, mongooses and bats
- Oral Rabies Vaccine (ORV) is a publicly acceptable methodology that may be applied on a broad geographic scale in specific wildlife reservoirs

Wildlife Rabies Control in Ontario

- ✓ **Between 1958 and 1990 there was an average of 1,500 confirmed cases of wildlife rabies per year.**
- ✓ **Since 1989 OMNR has used oral vaccines as a tool to control wildlife rabies. Program has been very successful.**
- ✓ **In 2009 there were only 16 confirmed cases in terrestrial mammals. 50% in skunks.**
- ✓ **Available oral rabies vaccines were ineffective in skunk.**
- ✓ **Control of rabies in wildlife remains an important challenge.**
- ✓ **A human adenovirus rabies glycoprotein recombinant vaccine (AdRG1.3) was developed for oral vaccination: *Yarosh et al. 1996, Vaccine***
- ✓ **AdRG1.3 rabies vaccine is produced by cell culture process using HEK 293 cell.**



Source: Ontario Ministry of Natural Resouces

- ➢ **Large quantity of vaccine is required for field trials to assess its efficacy.**

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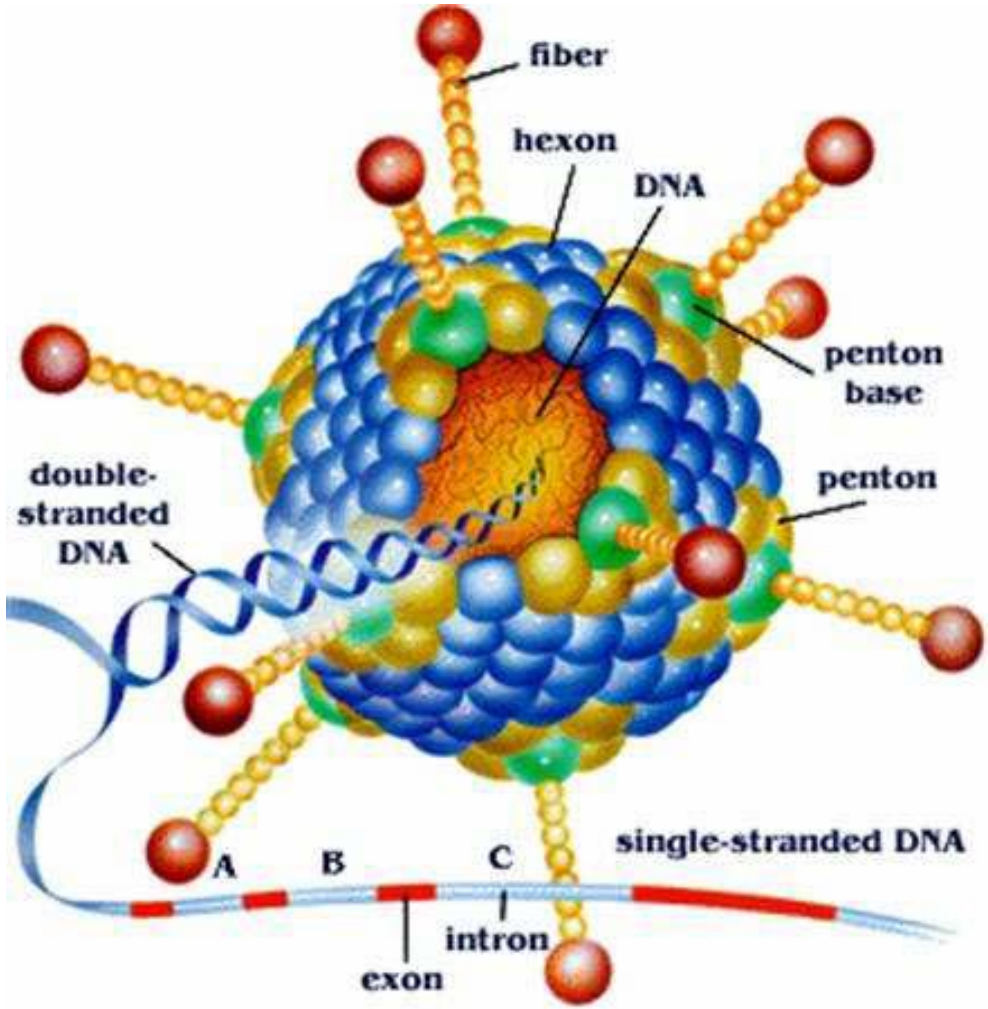
AdV vector

Vector

Well characterized
Broad tropism
Weakly pathogenic
Respiratory track

Manufacturing

High titers
High stability
Well characterized up- & downstream bioprocesses



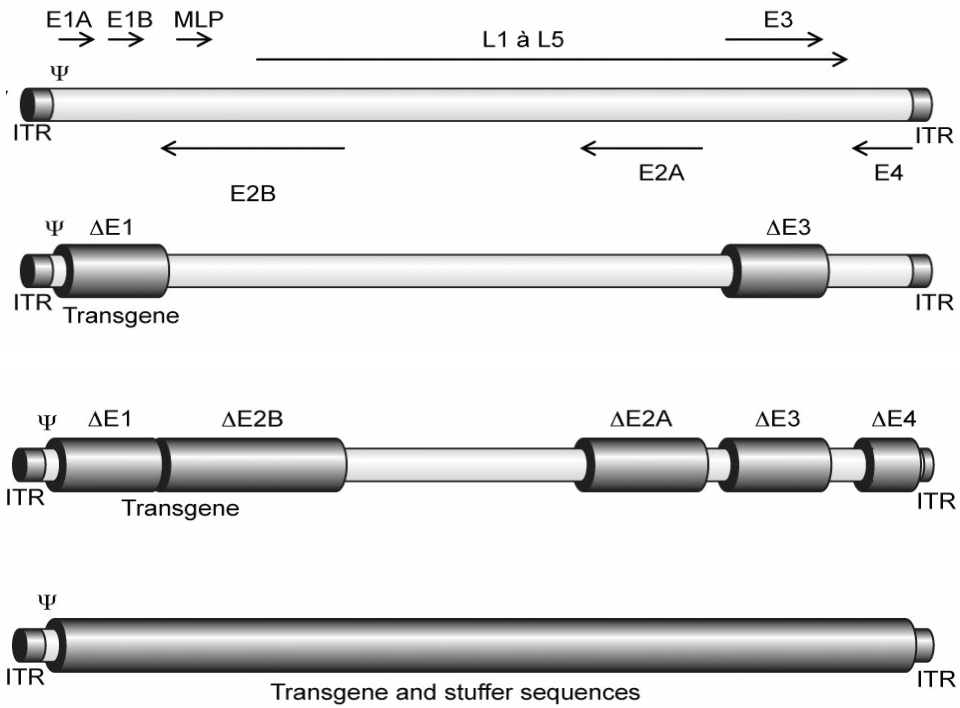
From first to third generation AdV

Wild-type AdV

1st generation

2d generation

3d generation



- ✓ Reduction of immunogenicity & toxicity
- ✓ Improved transgene capacity
- ✓ Lower production yield & increased complexity of production system

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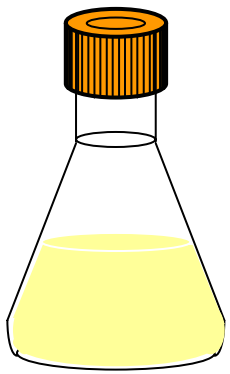
HEK293 a platform for viral vectors and vaccine manufacturing

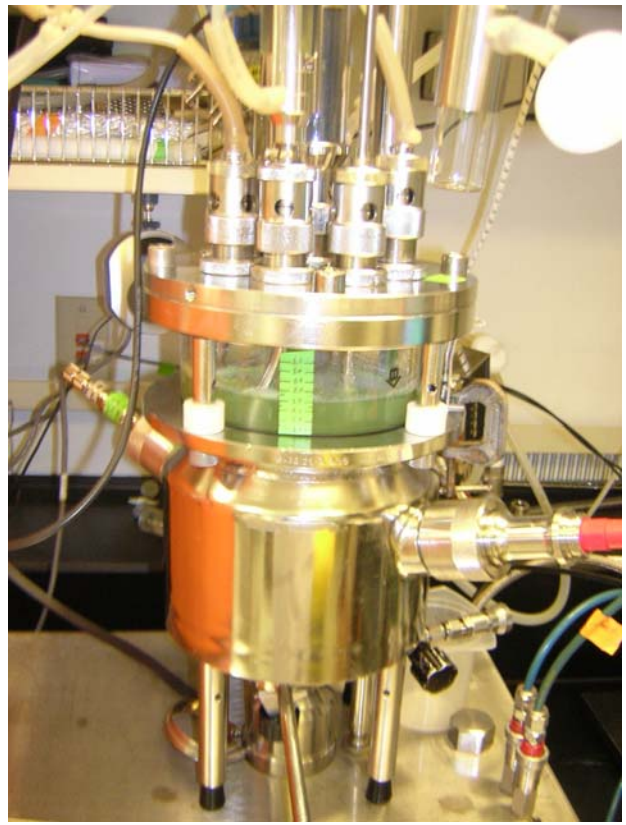
Production & Purification	Application	Scale	References
Adenovirus	vaccines	500L	Kamen et al., J. gene medicine, 2004 **P21**
Gutless AdV	Muscular Dystrophy	3L	Dormond et al., Biotechnol.Bioeng, 2009
AAV	Interferon	3L	Durocher et al., VirMet, 2007. **P9**
Retrovirus	Marker	3L, perfusion	Ghani et al., Biotechnol.Bioeng, 2006
Lentivirus	Marker	3L, perfusion	Ansorge et al. JGM 2009.
Reovirus	Oncolytic	100L	Transfiguration et al. J.PBA, 2008 **P21**
Influenza H1N1	Vaccine	3L	Le Ru et al. Vaccine, 2010 **P20**

Improvement of Production yield and Process Robustness

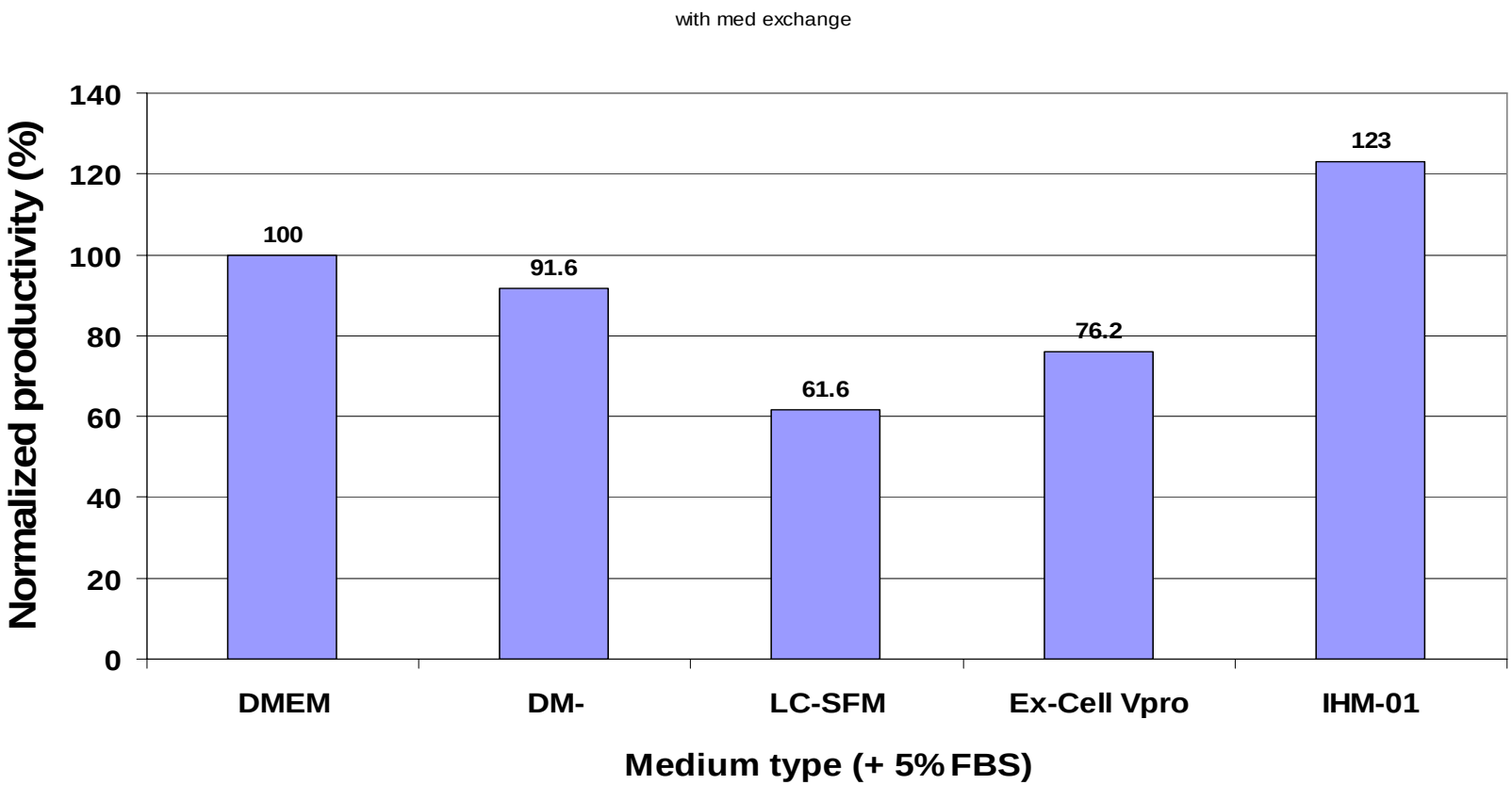
1. **Selection of a basal medium**
2. **Medium development**
3. **Increase in cell density**
4. **Streamlining the Process**

→ ***Transfer to bioreactor***





Selection of basal medium



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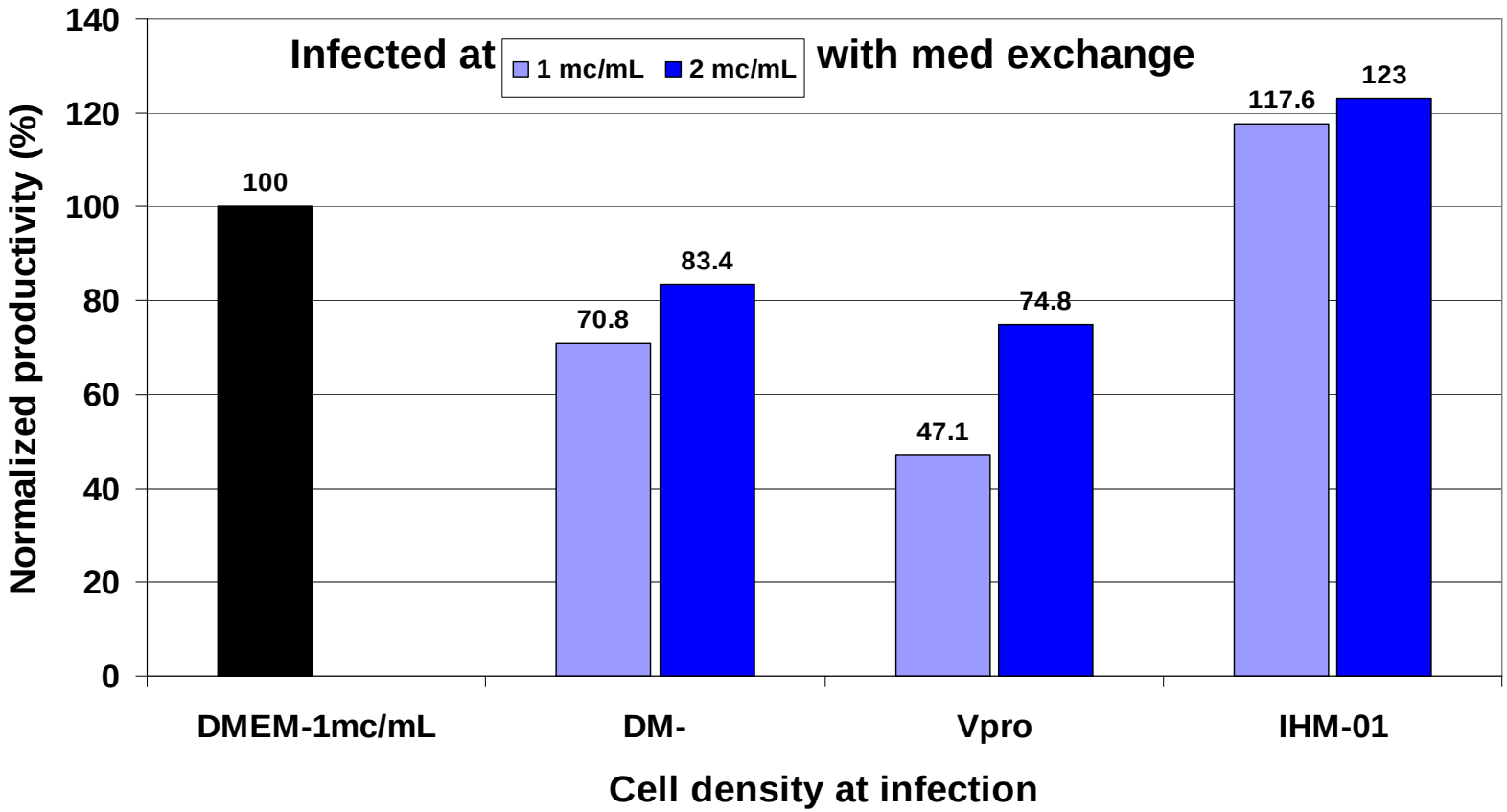
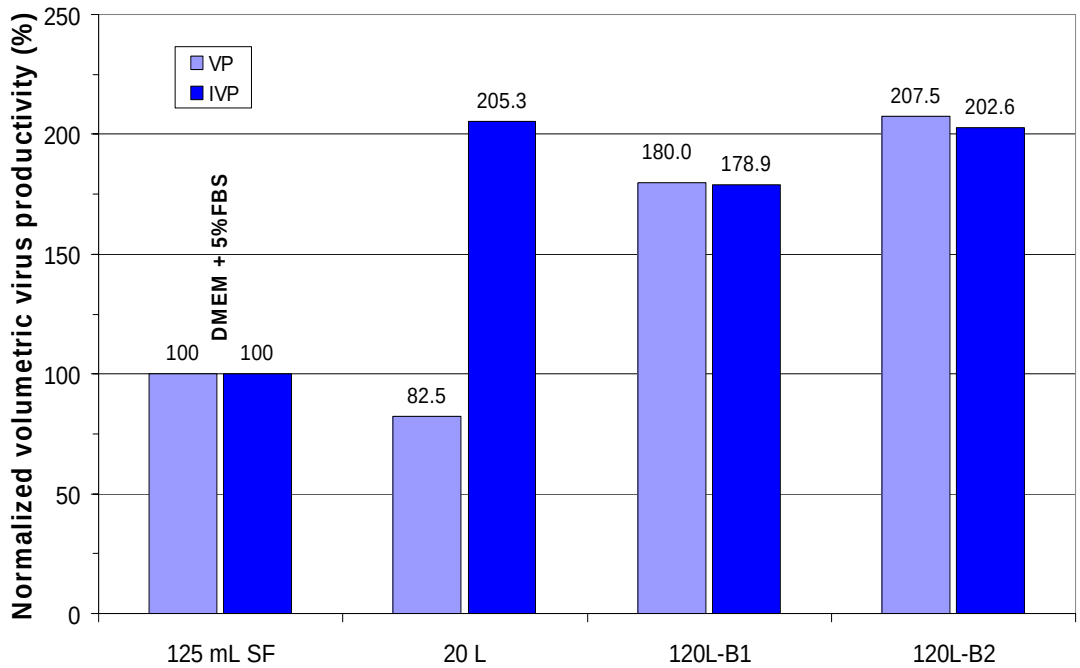
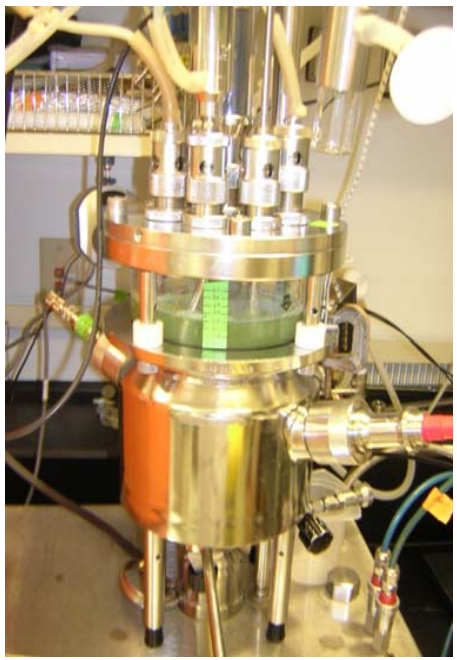
Effect of cell density at infection



Production of AdRG1.3 in 180 L bioreactor with medium exchange



More Robust Production Process for Manufacturing ?



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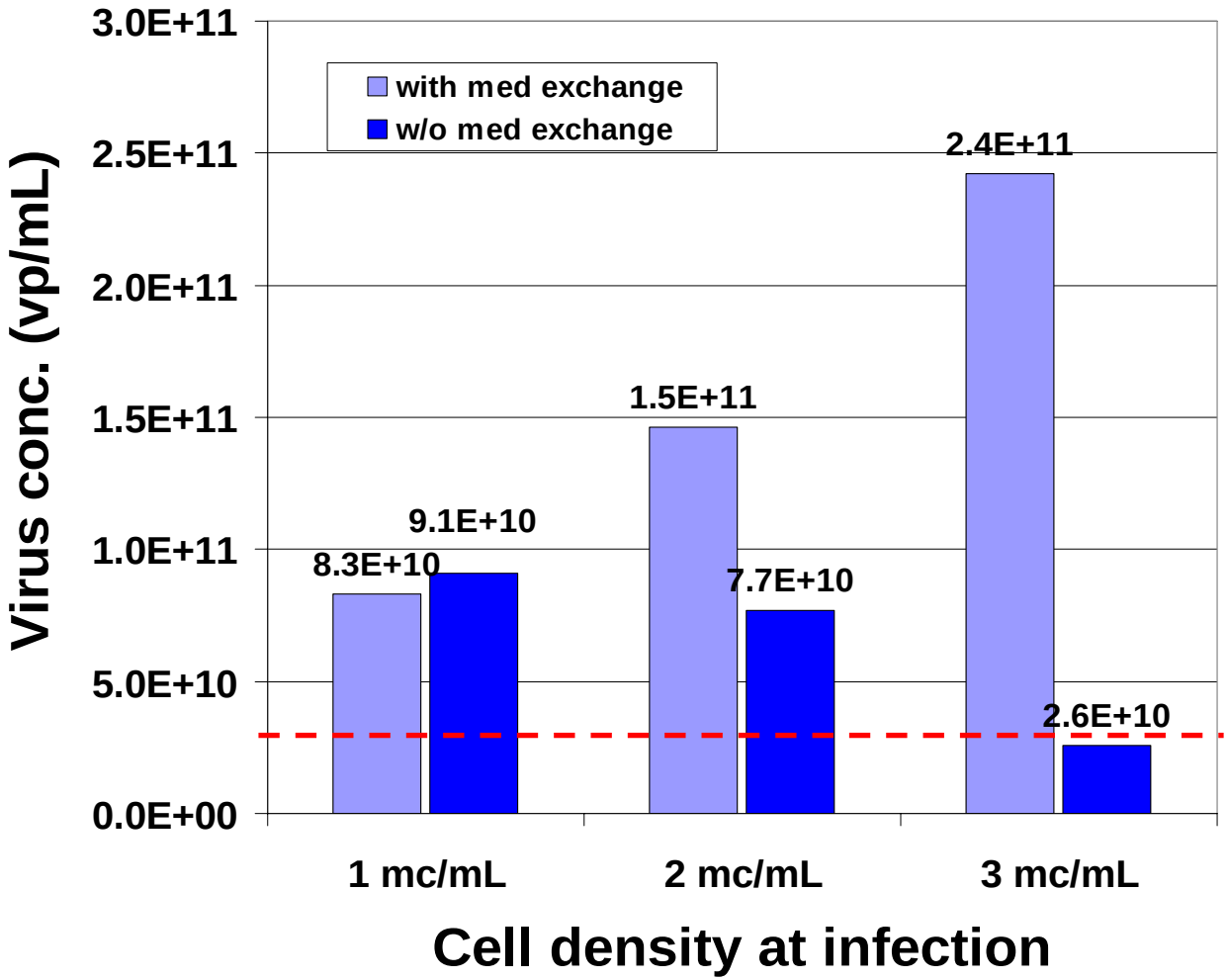
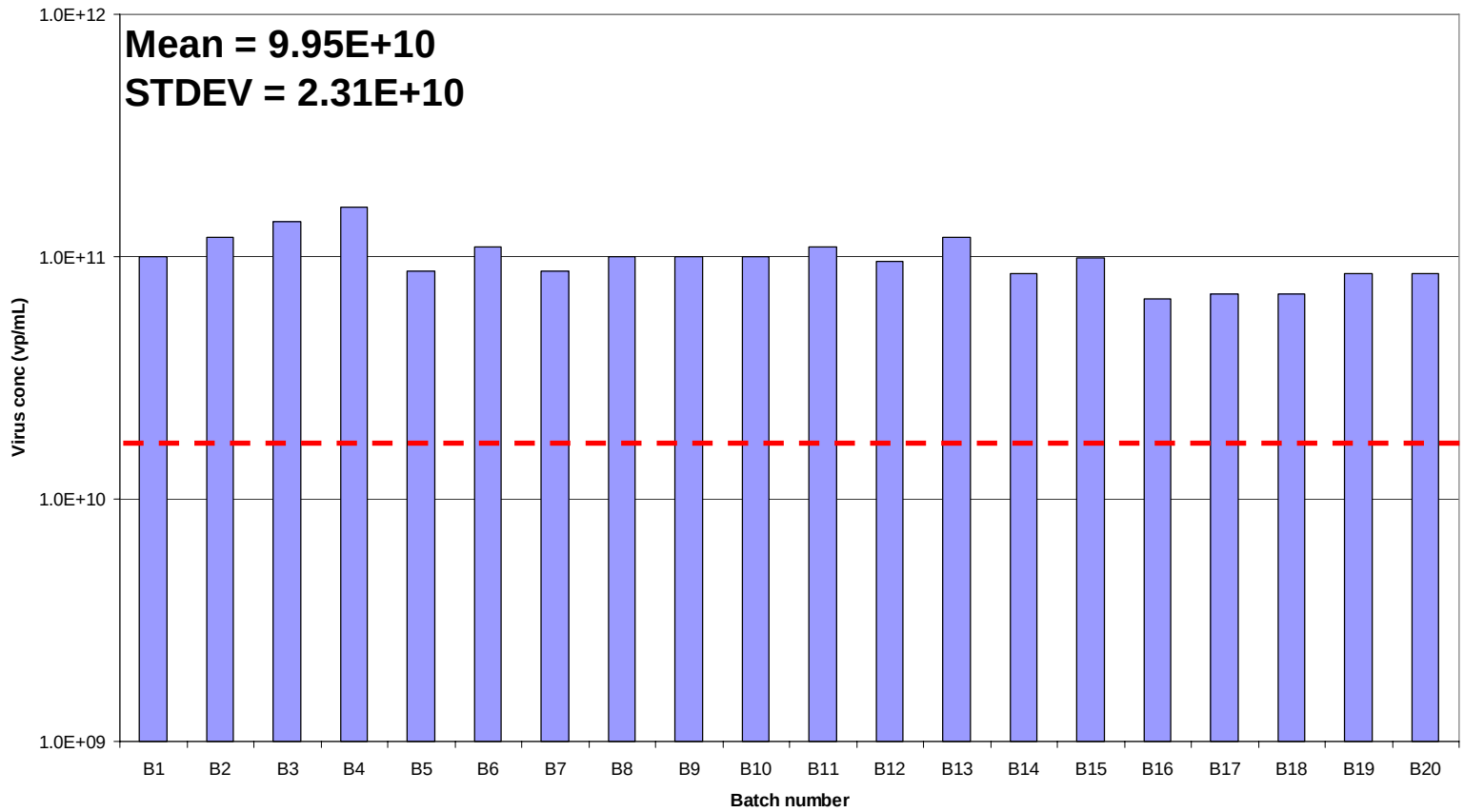
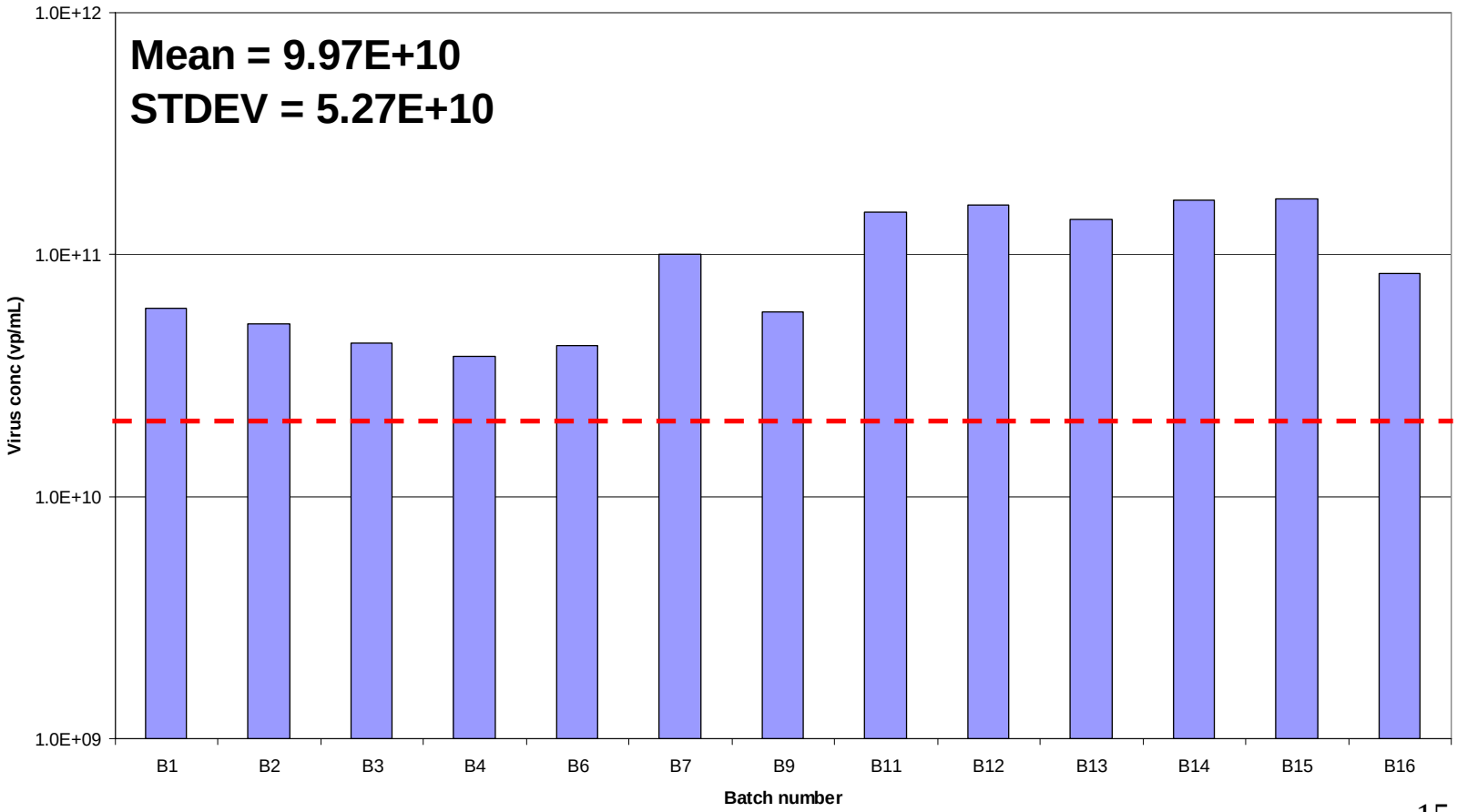
IHM-02, resulted in production w/o medium exchange



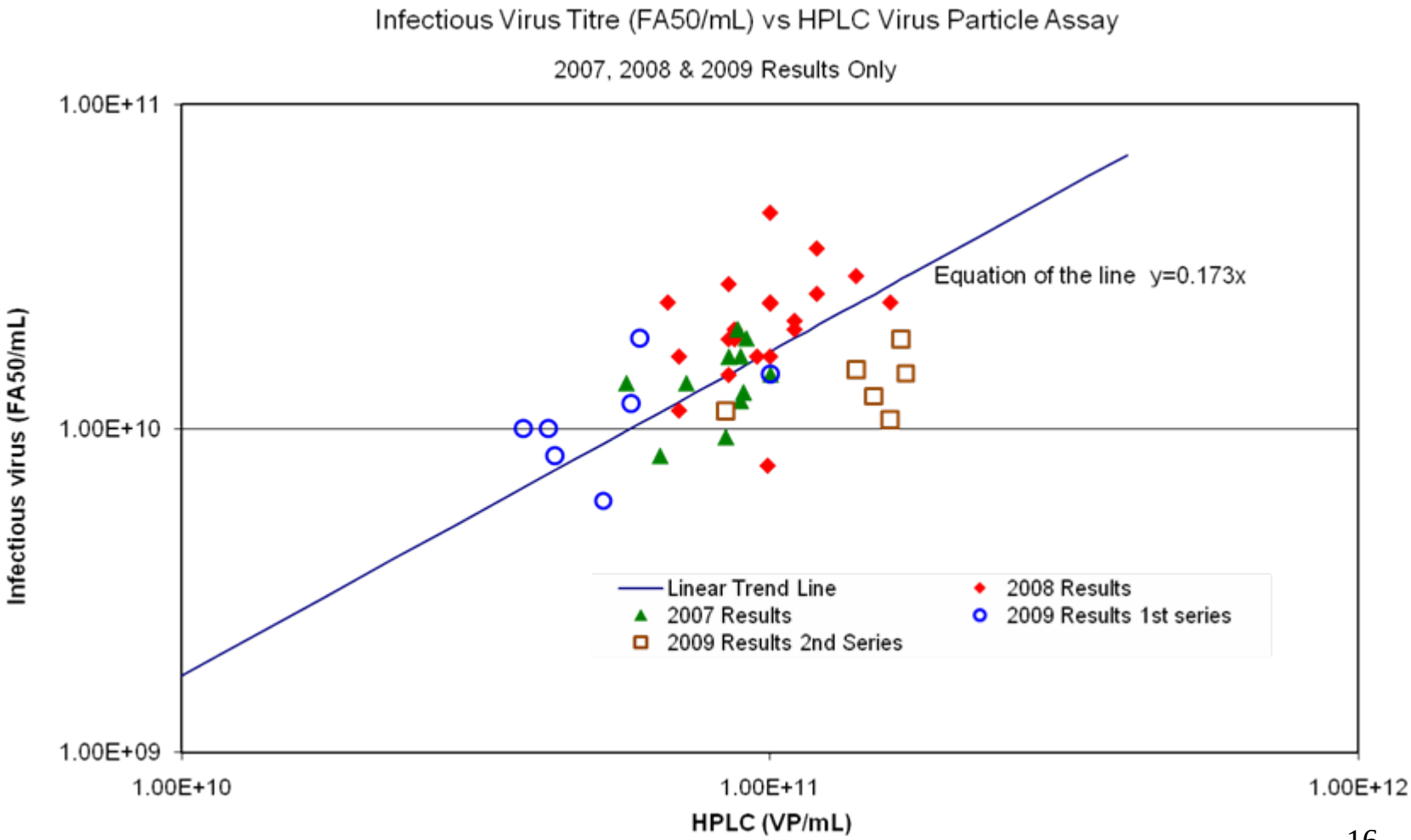
Production data 2008
160L batch



Production data 2009
500 L batch



Ratio IVP vs VP



Concluding Remarks: Process development

- **Improved production process resulting in**
  - **50% saving in material expenses and**
  - **25% reduction in labor costs**
  - **More robust process**
- **Improved AdRG1.3 viral yield by a factor of three through optimization of cell culture media**

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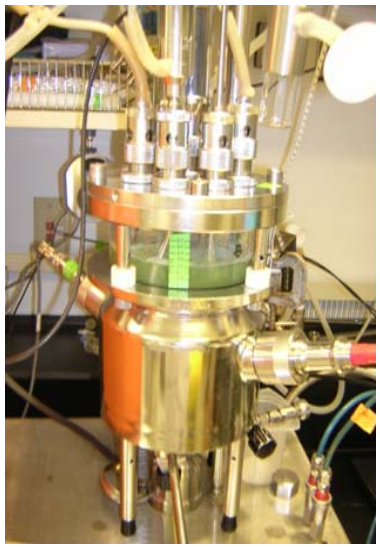
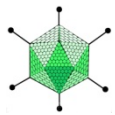
Successful Scale-up of AdV-based ORV vaccine



~ 12000L of AdRG1.3 (ONRAB) at 10^{11} VP/mL produced for field trials

ORV Baiting

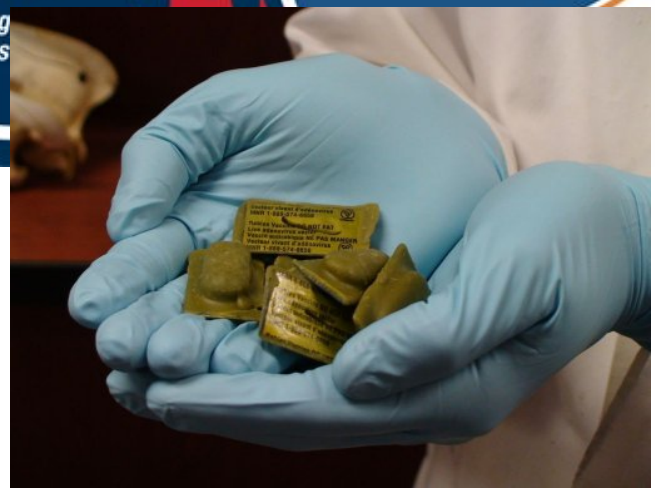
Vaccine-filled baits are distributed throughout the area by airplanes.



Key component in the baits is adenovirus type 5 expressing rabies glycoprotein (AdRG1.3).

Source: Ontario Ministry of Natural Resouces

Aerial baiting of rabies vaccine



Rabies vaccine bait developed by Ontario Ministry of Natural Resources

Baits will be distributed by plane.

Aerial baiting is part of Ontario's rabies control program.

Aerial baiting is done using a bait machine.

Sources: Ontario Ministry of Natural Resources

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Vaccine efficacy in Raccoons

TABLE 5. ONRAB® vaccine efficacy in raccoons in SW Ontario, Canada, that ate ONRAB baits in areas baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007.[a]

Bait density (baits/km^2)	ELISA+ (*n*)	ELISA− (*n*)	Sample size (*n*)	ELISA+ (%)
75	192	44	236	81.4
150	215	58	273	78.8
300	277	40	317	87.4
400	240	27	267	89.9
Total	924	169	1,093	

[a] Raccoons that ate ONRAB baits as determined by the presence of tetracycline in second premolar teeth; ELISA+ = ELISA positive; ELISA[minus] = ELISA negative; ELISA+ (%) = percentage of raccoon sera samples from raccoons that consumed a bait that were ELISA positive and is an indication of vaccine efficacy in raccoons.

Rosatte et al. (2009) Journal of Wildlife Diseases, 2009, 45:363-374

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Vaccine efficacy in Raccoons (2010)

New facilities for commercial manufacturing Artemis Technologies Inc.

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Conclusion

- Successful process optimization and scale-up for manufacturing of an adenovirus-base ORV
- Production of >> 5 millions baits for field trials
- Succesful technology transfer for manufacturing of adenovirus-based ORV
- Highly efficient ORV based on 2006/07 field trials
- This ORV-related program has potentially broad appplications in developing countries where most of the 55,000 human rabies cases per year are reported by WHO

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Vaccine 27 (2009) 2662–2668

Contents lists available at ScienceDirect

Vaccine

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

In vitro and *in vivo* genetic stability studies of a human adenovirus type 5 recombinant rabies glycoprotein vaccine (ONRAB)

M. Kimberly Knowles*, Danielle Roberts[1], Sheona Craig, Mary Sheen, Susan A. Nadin-Davis, Alexander I. Wandeler

Vaccine 27 (2009) 6619–6626

Contents lists available at ScienceDirect

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Safety studies on an adenovirus recombinant vaccine for rabies (AdRG1.3-ONRAB®) in target and non-target species

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