

## NRC Publications Archive Archives des publications du CNRC

### Process scale-up and optimization for production of high efficacy oral rabies vaccine

Lamen, Amine; Shen, Chun Fang; Lanthier, Stephane; Beresford, Andrew; Montes, Johnny; Beresford, Andrew

For the publisher's version, please access the DOI link below./ Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

<https://doi.org/10.4224/23001977>

**NRC Publications Archive Record / Notice des Archives des publications du CNRC :**  
<https://nrc-publications.canada.ca/eng/view/object/?id=ef506fef-5eaf-4fa2-a15f-e82157388ff2>  
<https://publications-cnrc.canada.ca/fra/voir/objet/?id=ef506fef-5eaf-4fa2-a15f-e82157388ff2>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at  
<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site  
<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

**Questions?** Contact the NRC Publications Archive team at  
PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

**Vous avez des questions?** Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

CMRC-NRC



Institut de  
recherche en  
biotechnologie

NRC-CMRC



Biotechnology  
Research  
Institute



# ***Process Scale-up and Optimization for Production of High Efficacy Oral Rabies Vaccine***

***Amine Kamen***

***VT III, Nuevo Vallarta, Mexico June 9, 2010***



Conseil national  
de recherches Canada

National Research  
Council Canada

Canada

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

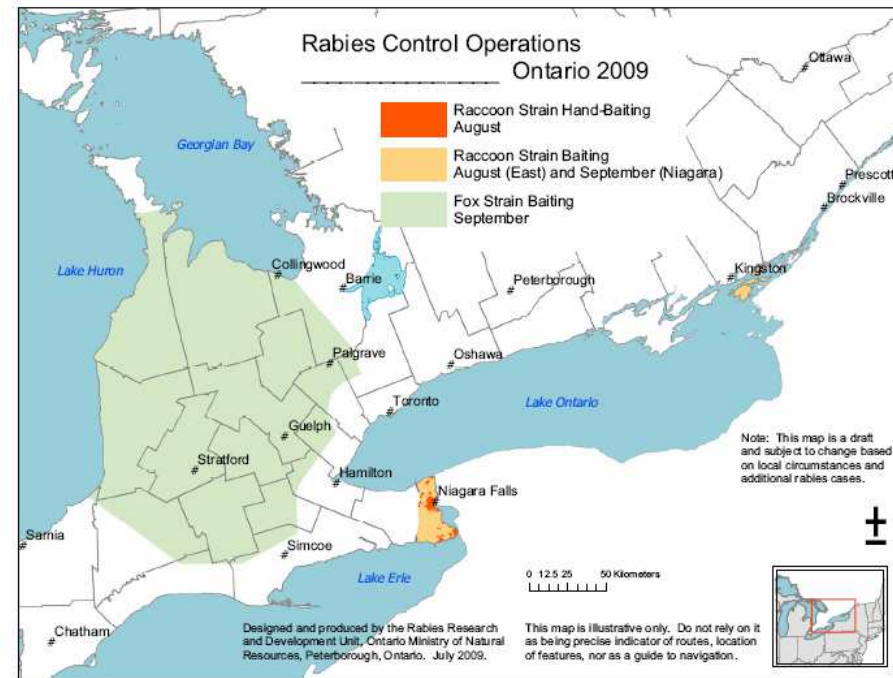


# *Rabies: Old Disease New Challenges*

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



Source: Ontario Ministry of Natural Resources

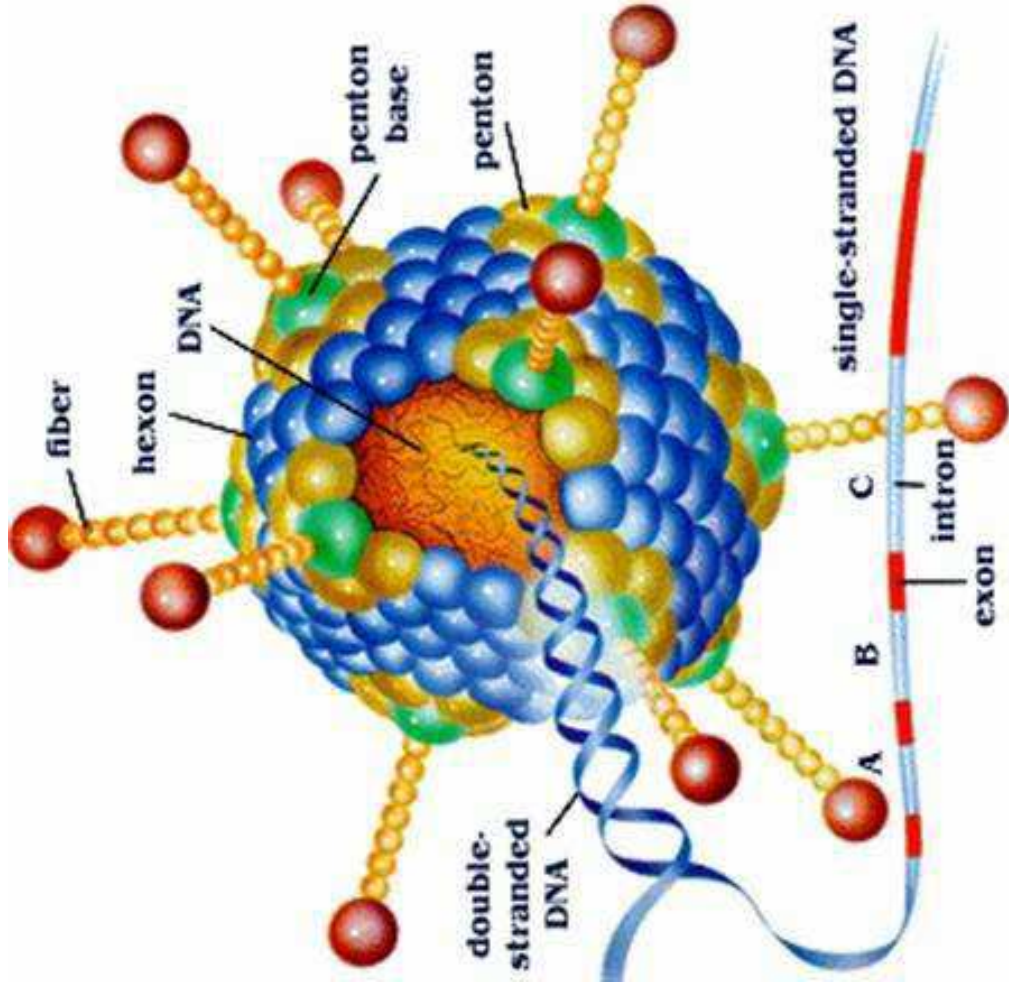


## Vector

- 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



1<sup>st</sup> generation



2<sup>d</sup> generation



3<sup>d</sup> generation



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

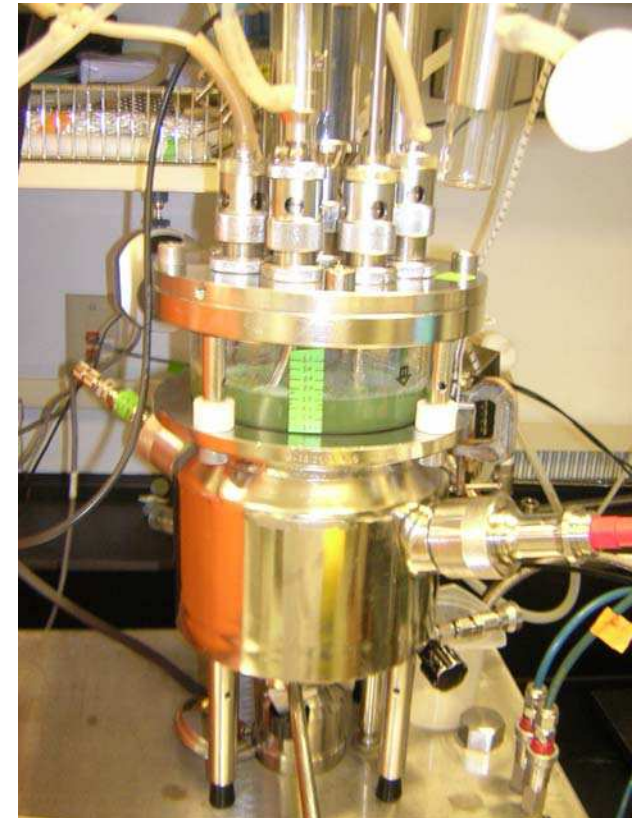
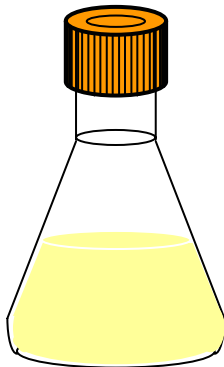
# HEK293 a platform for viral vectors and vaccine manufacturing

Production & Purification	Application	Scale	References
Adenovirus	vaccines	500L	Kamen et al., J. gene medicine, 2004 <b>P21</b>
Gutless AdV	Muscular Dystrophy	3L	Dormond et al., Biotechnol.Bioeng, 2009
AAV	Interferon	3L	Durocher et al., VirMet, 2007. <b>P9</b>
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 <b>P21</b>
Influenza H1N1	Vaccine	3L	Le Ru et al. Vaccine, 2010 <b>P20</b>



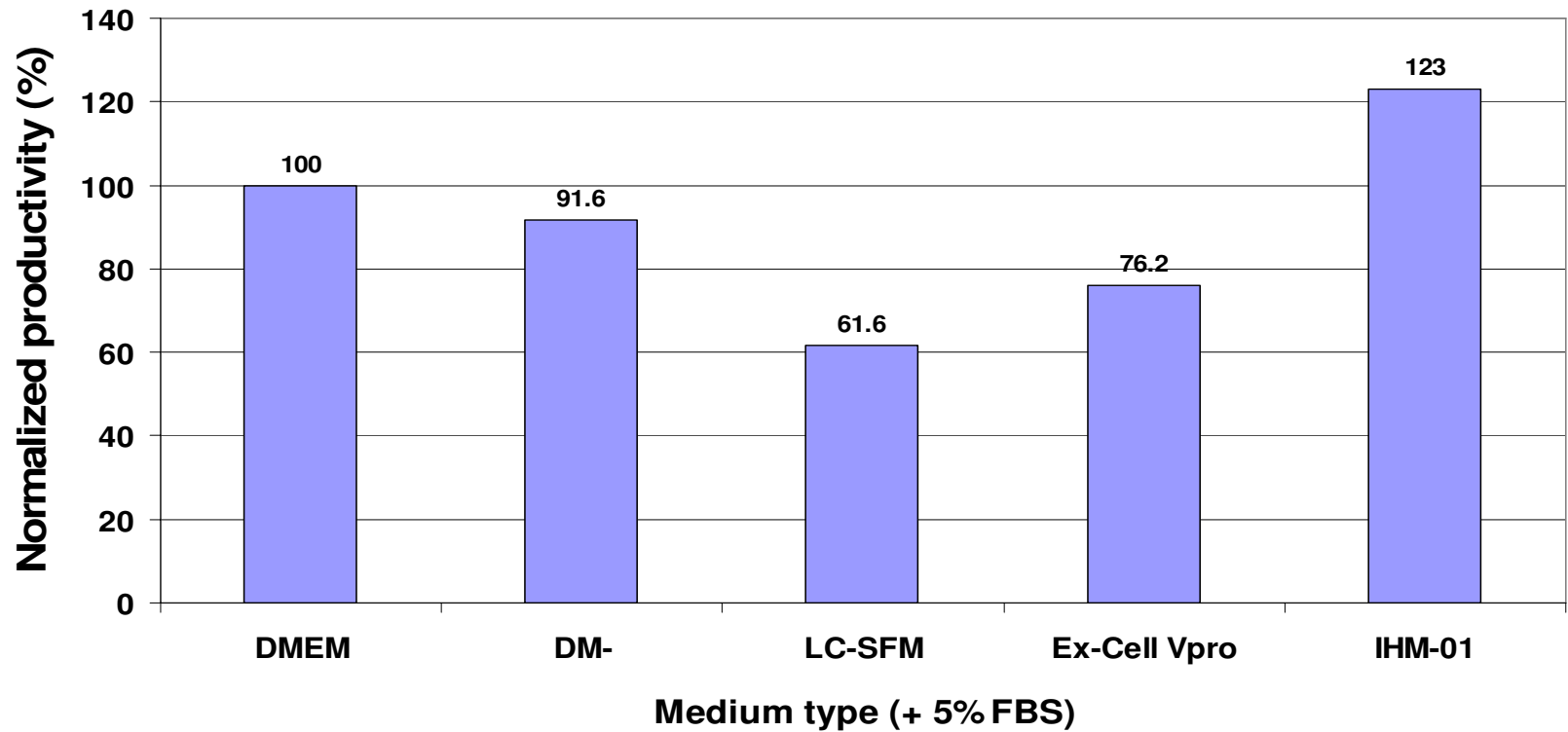
# *Improvement of Production yield and Process Robustness*

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

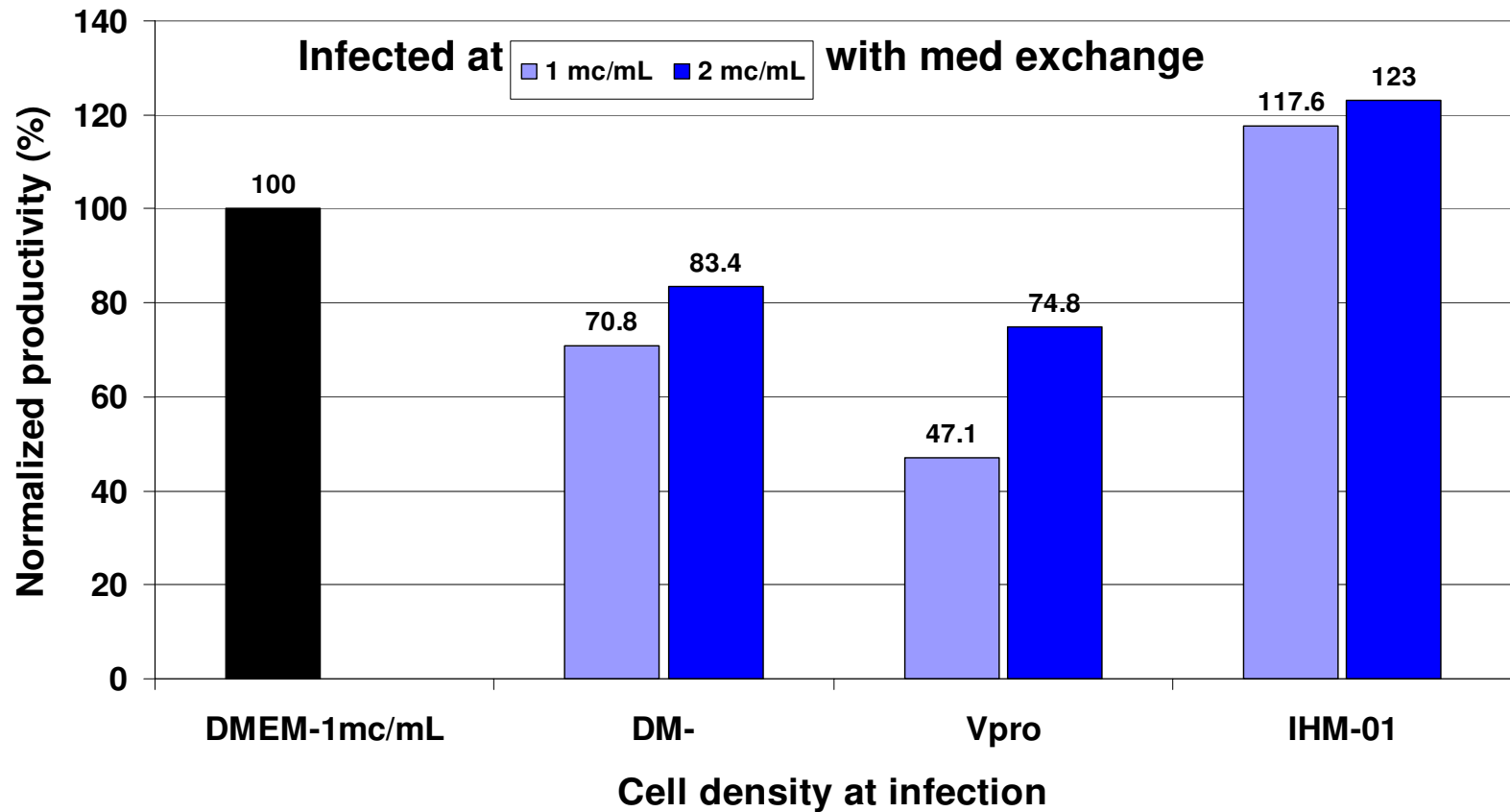


# Selection of basal medium

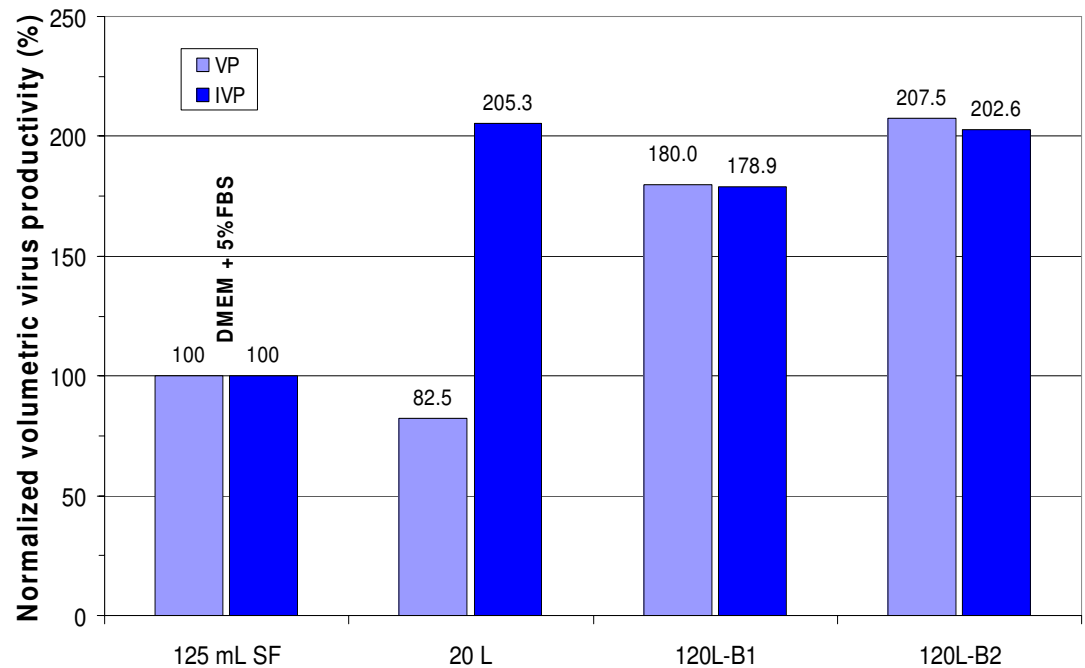
with med exchange



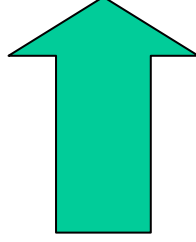
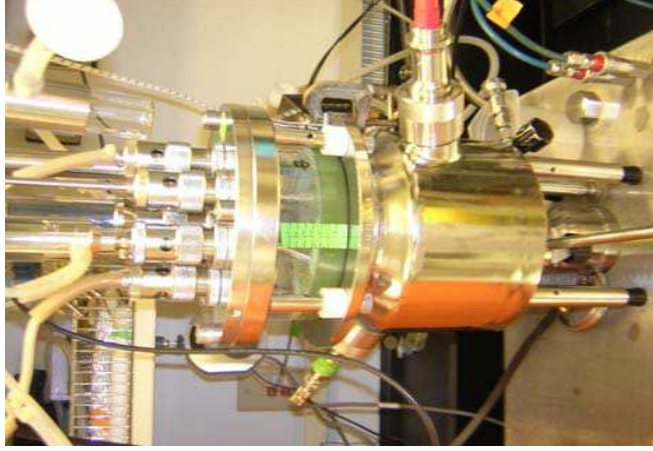
# Effect of cell density at infection



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

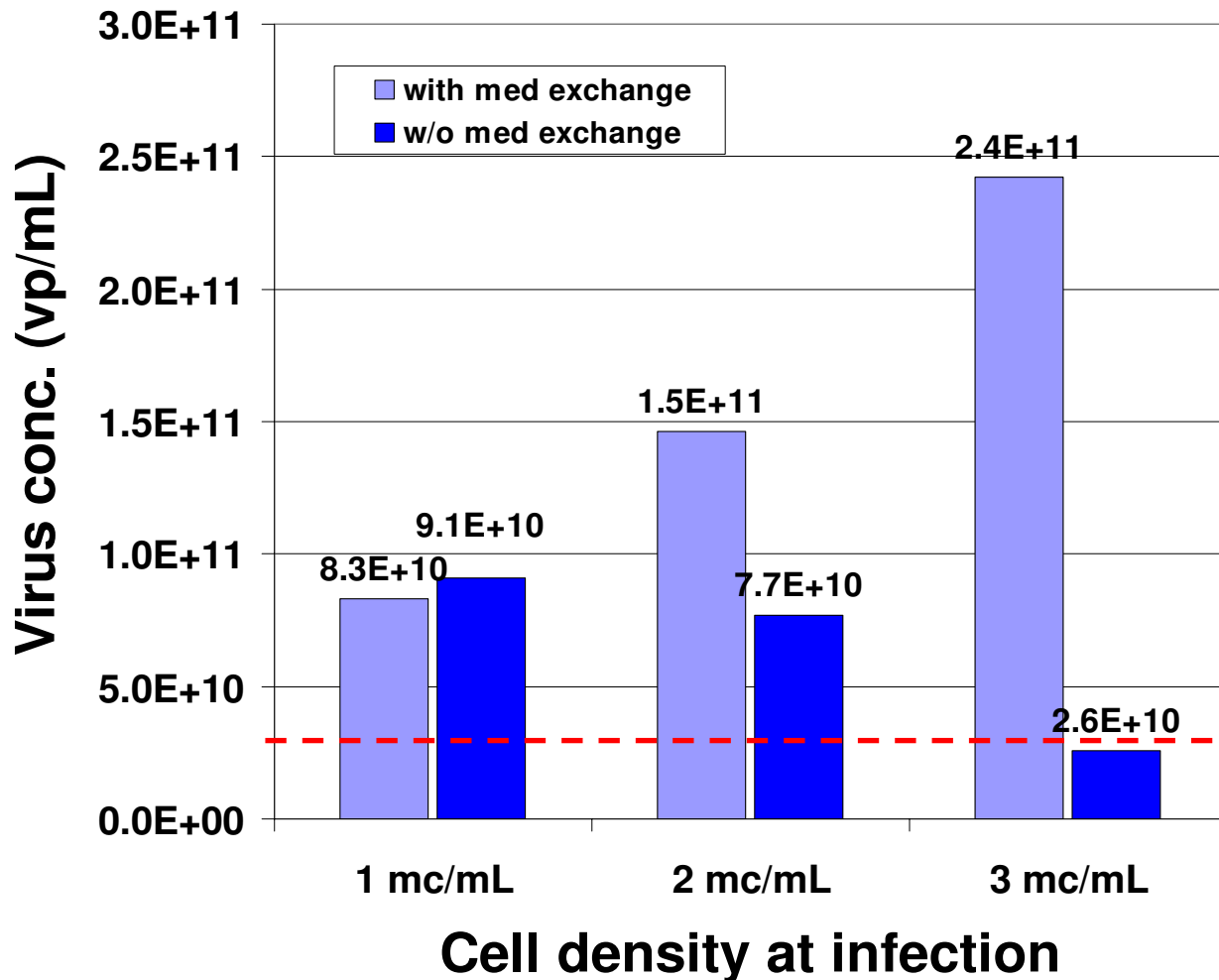


# *More Robust Production Process for Manufacturing ?*



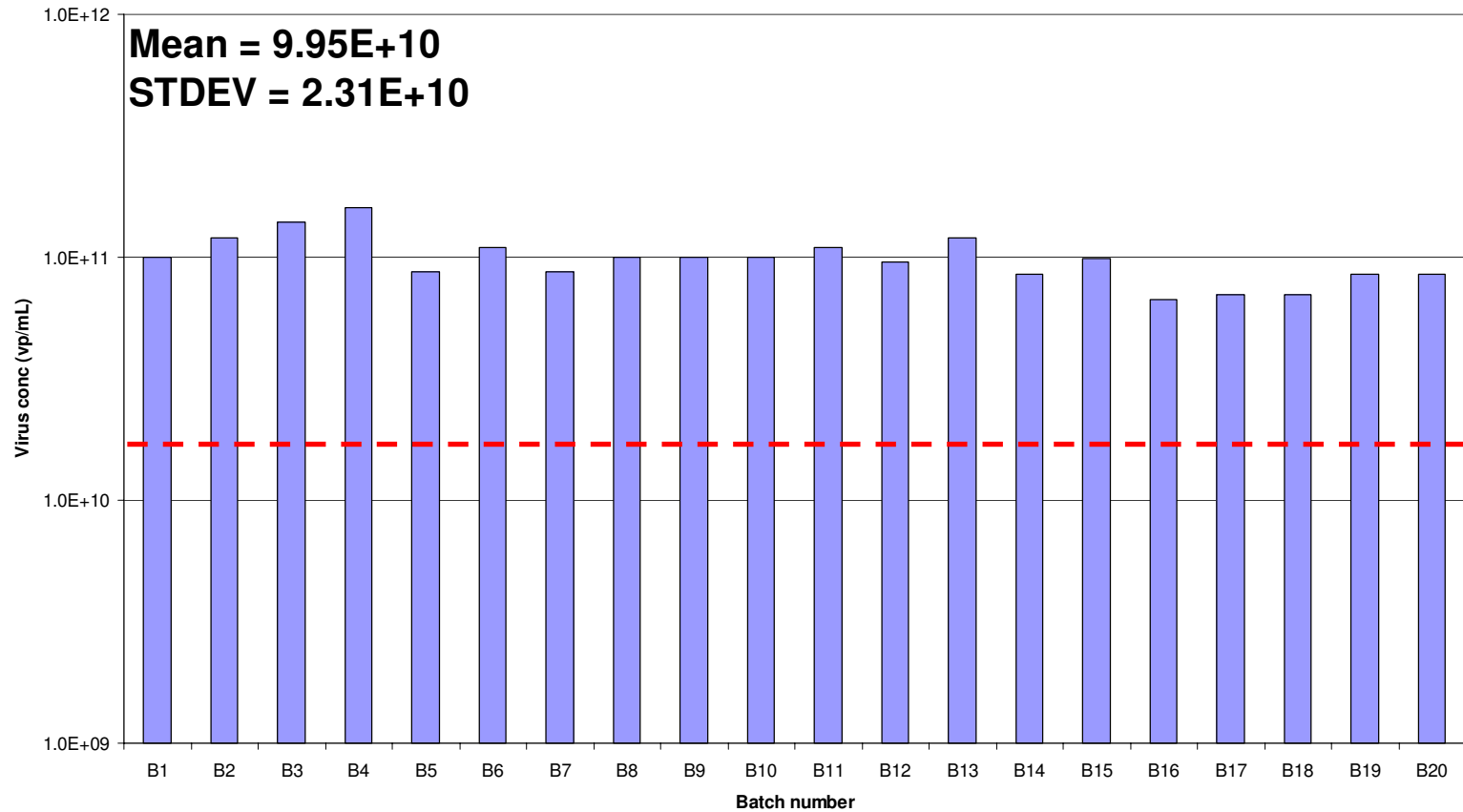


*IHM-02, resulted in  
production w/o  
medium exchange*



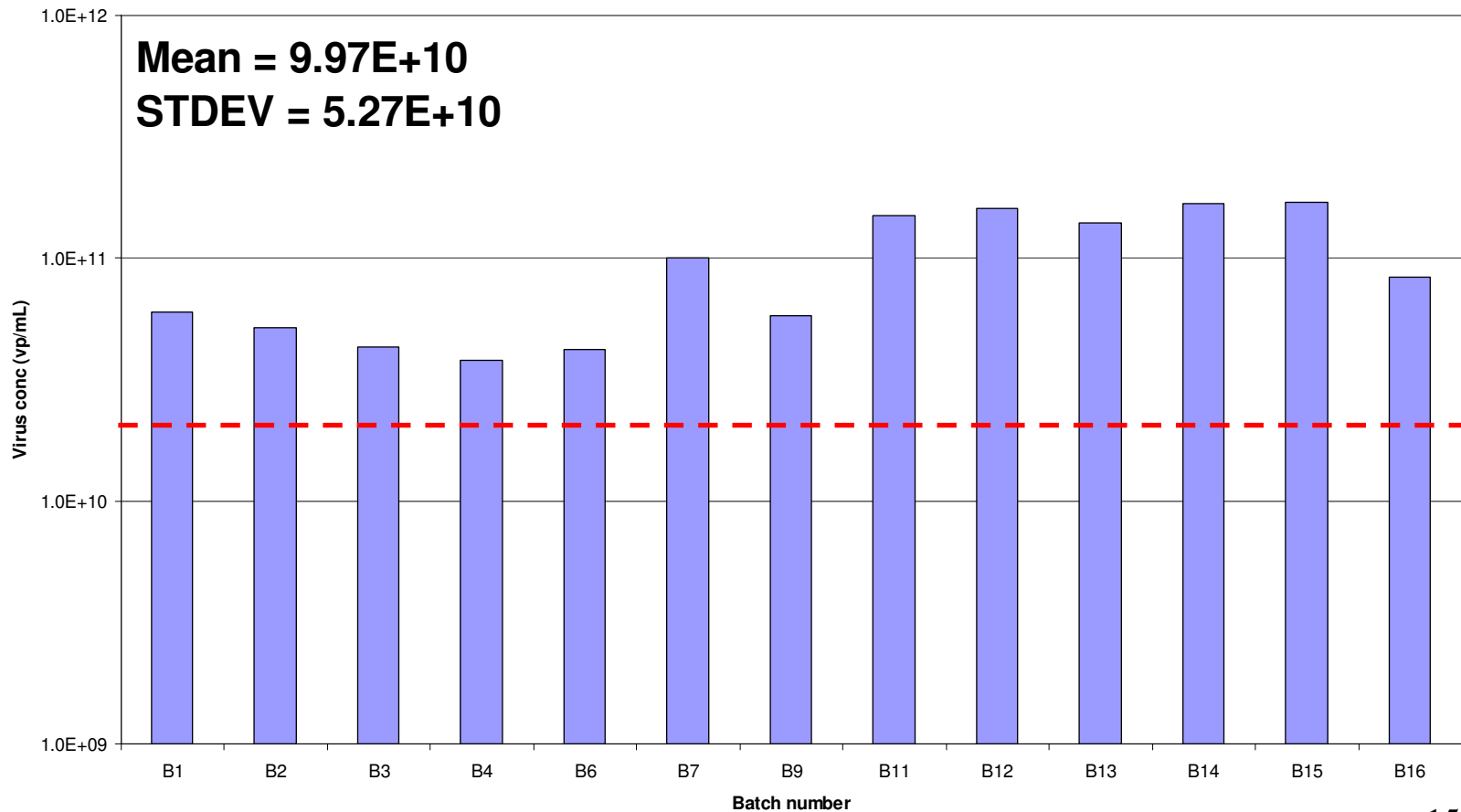
# Production data 2008

## 160L batch



# Production data 2009

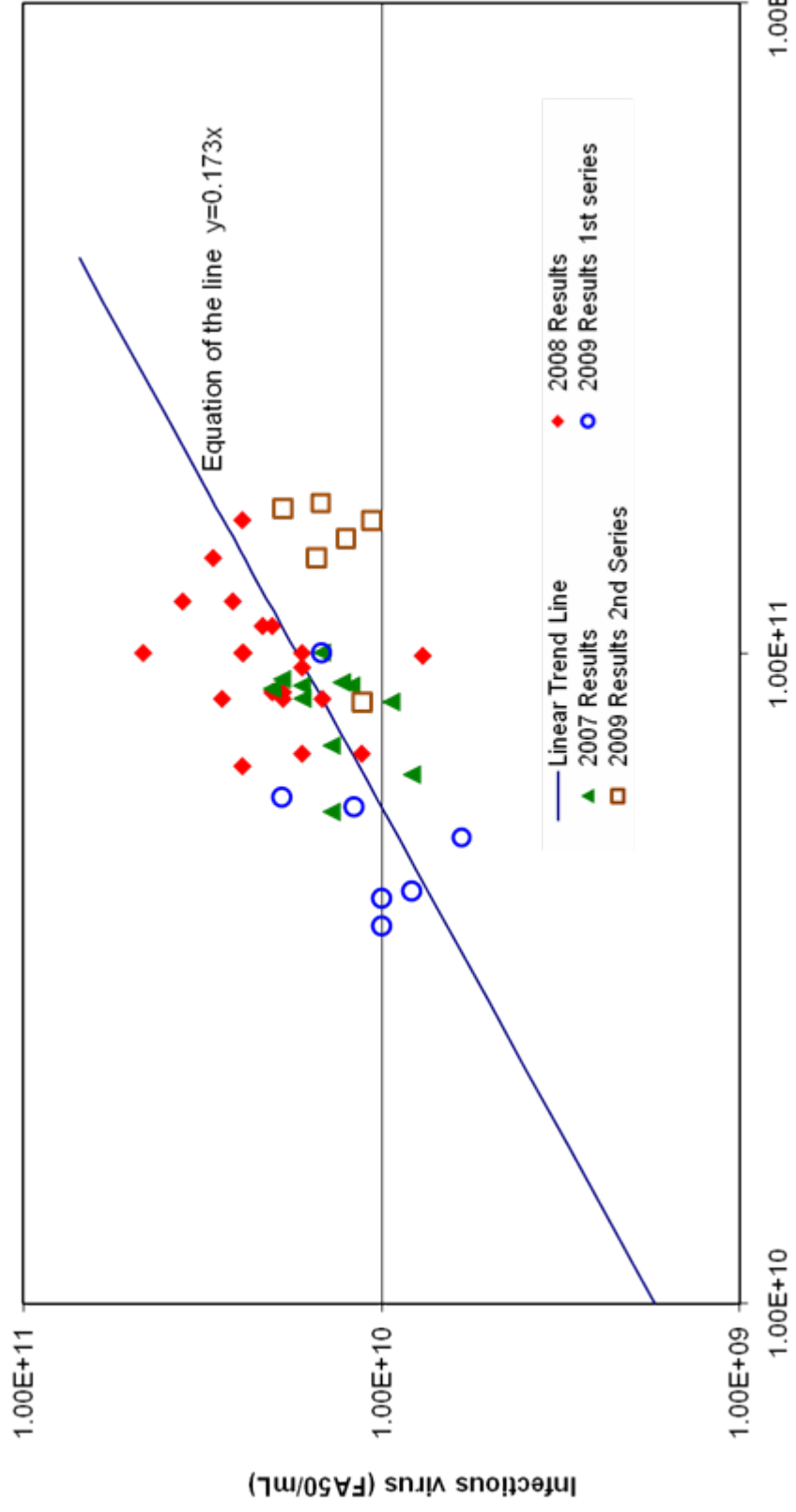
## 500 L batch



# Ratio IVP vs VP

Infectious Virus Titre (FA50/mL) vs HPLC Virus Particle Assay

2007, 2008 & 2009 Results Only

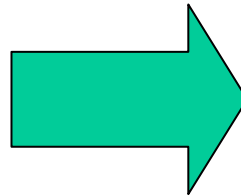
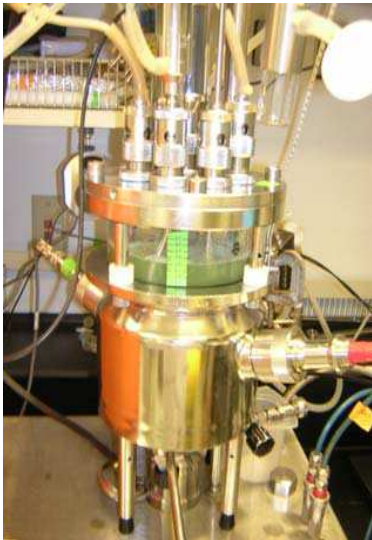


## *Concluding Remarks: Process development*

- **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**



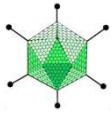
# Successful Scale-up of AdV-based ORV vaccine



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



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



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





**Rabies vaccine bait developed by Ontario  
Ministry of Natural Resources**

# *Aerial baiting of rabies vaccine*



**Baits will be distributed by plane.**



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



**Aerial baiting is done using a bait machine.**

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<sup>2</sup> during 2006 and 2007.<sup>a</sup>

Bait density (baits/km <sup>2</sup> )	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	

<sup>a</sup> 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.



## The rabies reporter

MNR Publication 51709 Volume 21, Number 1

January - March 2010

### Rabies in the first quarter: (almost) nothing to report

*Beverly Stevenson*

There isn't much that can be said about the rabies cases in Ontario during the first three months of 2010. This is largely because there was only one (no that's not a typo) confirmed case of rabies in the province during this quarter.



***New facilities for  
commercial  
manufacturing  
Artemis  
Technologies Inc.***



## *Conclusion*

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

## **Project Team**

**Chun Fang Shen**

**Stephane Lanthier**

**Danielle Jacob**

**Johnny Montes**

**Olivier Henri**

***Hélène Coelho***

***Robert Voyer***

***Ricardo Ochoa***

***Louis Bisson***

***Alice Bernier***

## **Artemis Technologies**

**Andrew Beresford**

**Alex Beath**

## **Other Departments and Agencies**

**OMNR**

**CFIA**