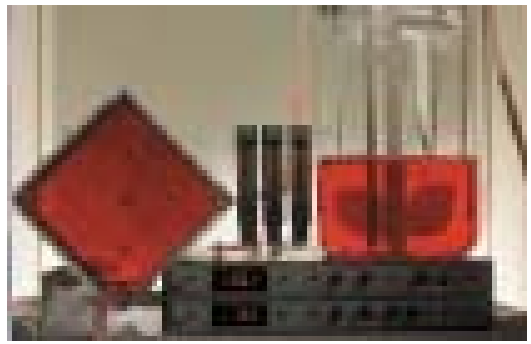
BioReliance®
invitrogen bioservices

Assays for Bovine Viruses

Dr Martin Wisher

Director for Market Development and Regulatory Affairs



BioReliance®
invitrogen bioservices

Testing of Bovine Raw Materials

- US Code of Federal Regulations
  - 9CFR 113.53 Requirements for ingredients of animal origin used for production of biologics.
  - 9CFR 113.46 Detection of cytopathogenic and/or hemadsorbing agents
  - 9CFR 113.47 Detection of extraneous viruses by the fluorescent antibody technique
- EMEA CPMP Note for Guidance on the Use of Bovine Serum in the Manufacture of Human Biological Medicinal Products. CPMP/BWP/1793/02
- European Pharmacopoeia Draft Monograph ‘Bovine Serum’ (2003)

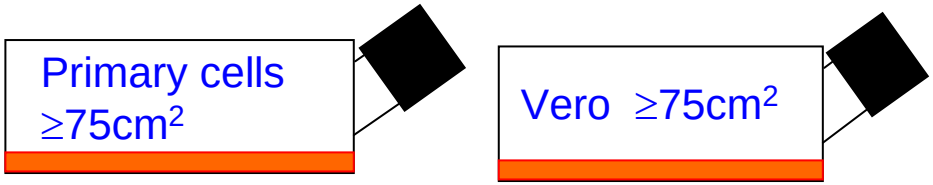
BioReliance®
invitrogen bioservices

9 CFR Bovine Virus Assays

- General tests for:
  - Cytopathogenic agents
  - Haemadsorbing agents

- Specific test (fluorescent antibody) for:
  - Bovine viral diarrhoea virus (BVDV)
  - Blue tongue (BTV)
  - Bovine adenovirus (BAV)
  - Bovine parvovirus (BPV)
  - Bovine respiratory syncytial virus (BRSV)
  - Reovirus 3
  - Rabies

Testing according to 9CFR Part 113.53



Cells are prepared in medium containing 3.75ml or 15% of the test ingredient

≥ 21days

Monolayers prepared for endpoint assays (each ≥6cm²).
Monolayers also prepared for IF positive control.

≥ 7days after last passage, test for:

-cytopathogenic agents
-hemadsorbing agents
-IF for BVDV, BTV, BAV, BPV, BRSV, *Reovirus*, *Rabies*

BioReliance®
invitrogen bioservices

Testing to CPMP Note for Guidance

- Tests for viral contaminants (prior to inactivation)

- General tests for
  - Haemadsorbing viruses (e.g., PI3)
  - Cytopathic agents (e.g., IBRV)

- Specific tests for:
  BTV, BAV, BPV, BRSV, BVDV, Reo3, Rabies

Testing for BVDV

CPMP Note for Guidance recommendations for BVDV

- Presence of BVDV in serum cannot be completely avoided
- Test for BVDV before inactivation, and if detected titrate virus. Test for BVDV again after inactivation when no infectious virus should be detected.

BioReliance®
invitrogen bioservices

Testing for BVDV Antibodies

- Validated test for anti-BVDV antibodies should be conducted

- Serum should be free of BVDV antibodies or at a level that does not interfere with the detection of BVDV

- Comparative titration tests
  - prepare cell cultures in media with test serum
  - subculture at least 3 times using media supplemented with test serum
  - use cells in a titration of a reference control BVDV stock

- High antibody titres can be detected

BioReliance®
invitrogen bioservices

Bovine Polyoma virus

The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 18 June 2003
CPMP/BWP/1793/02

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

NOTE FOR GUIDANCE ON THE USE OF BOVINE SERUM IN THE MANUFACTURE OF HUMAN BIOLOGICAL MEDICINAL PRODUCTS

DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY	September 2001 – March 2002
TRANSMISSION TO CPMP	March 2002
RELEASE FOR CONSULTATION	April 2002
DEADLINE FOR COMMENTS	October 2002
DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY	November 2002- June 2003
TRANSMISSION TO CPMP	June 2003
ADOPTION BY CPMP	July 2003
DATE FOR COMING INTO OPERATION	1 October 2003

"Methods based on the detection of viral DNA suggest that Bovine Polyoma virus is a common contaminant of bovine serum.
Serum manufacturers and users are encouraged to apply infectivity assays for BPyV and to investigate methods for inactivation/ removal of BPyV in order to limit or eliminate infectious virus from batches of serum"

BioReliance
invitrogen bioservices

Facts about Bovine Polyoma Virus (1)

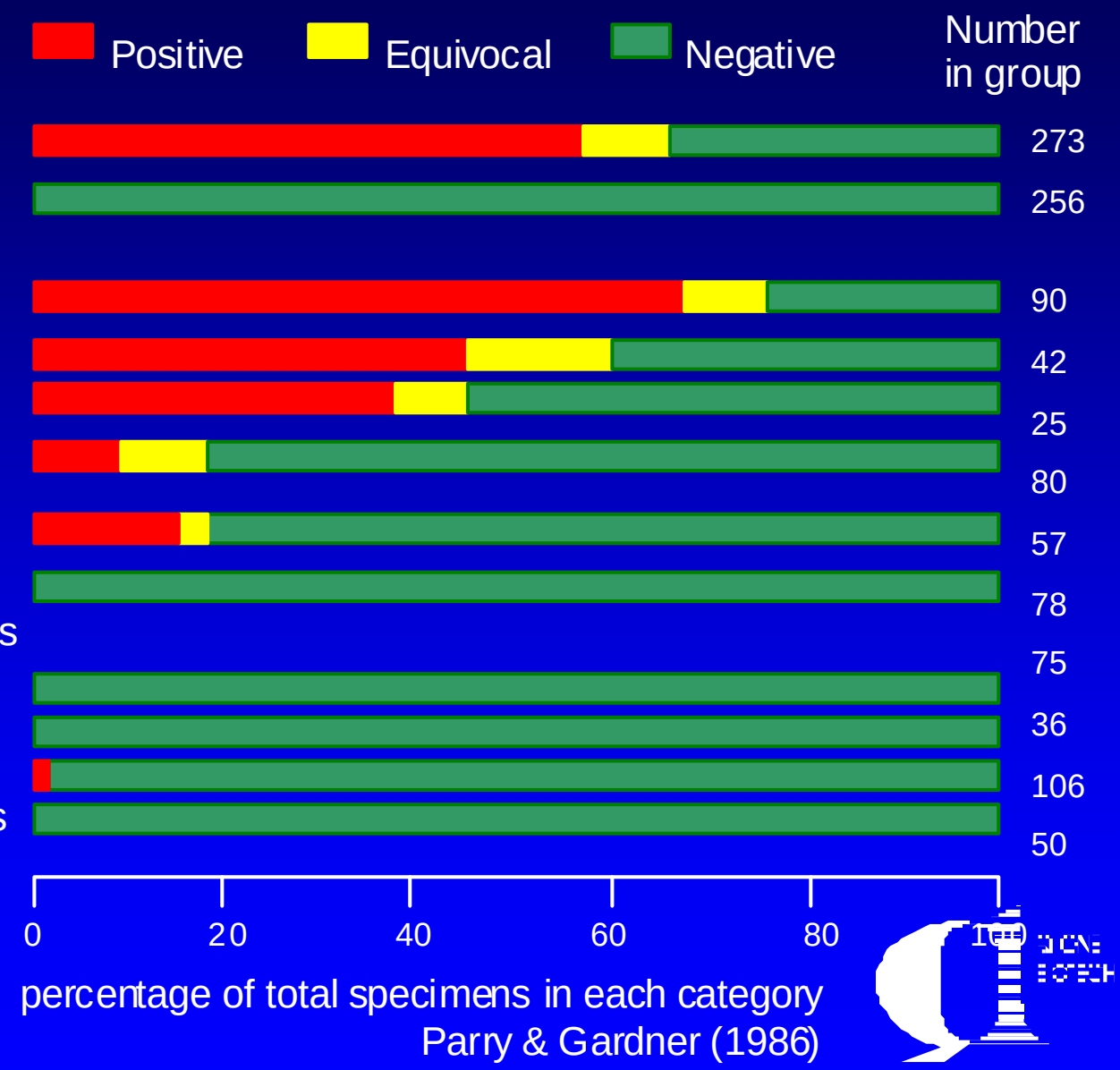
- Member of small DNA tumour virus family
  - ability to transform established cell lines
  - immortalise primary cell cultures
  - induce tumours in animals
- BPyV - small circular DNA genome of 4697bp (SV-40, 5243bp) (Schuurman *et al*., 1990)
- Constructs containing early region of BPyV under control of the RSV promoter capable of inducing tumour formation in rodent cells following transfection (Schuurman *et al*., 1992)

BioReliance®
invitrogen bioservices

Facts about Bovine Polyoma Virus (2)

- Virus isolated from kidney cell cultures of stump-tailed macaque (Wognum *et al*., 1984) and from foetal rhesus monkey kidney cell lines (Parry *et al*., 1983)
- No viral antibodies present in monkey hosts
- High proportion of cattle shown to be positive for antibody to the virus
- Individuals who had been in close contact with cattle possessed antibodies to the virus -zoonotic potential of virus? (Parry & Gardner, 1986)

Antibody to BPyV in Humans



BioReliance
invitrogen bioservices

Facts about BPyV (3)

- Evidence suggested that virus was of bovine origin and that primate cultures had become infected from use of contaminated bovine serum in culture medium

- Studies have shown that approximately 70% of bovine serum batches are positive for BPyV by PCR (Schuurman *et al*., 1991, Kappeler *et al*., 1996)

- BioReliance in-house testing found 90% of serum samples to be positive by conventional PCR
  - 71 batches, 7 negative
  - USA, Australia, New Zealand, South America, Europe

BioReliance
invitrogen bioservices

Infectivity Assay for Bovine Polyoma Virus

- MDBK cells used as detector cells
- No visible cytopathic effect. Polyoma viruses are not released by cell lysis
  - Q-PCR endpoint required
- Cells inoculated with test article for 2-3 hours. Cells maintained in low serum concentrations with passage at least every 2 weeks
- Culture period 8 weeks. Test cell supernatants by Q-PCR at weeks 1 and 8

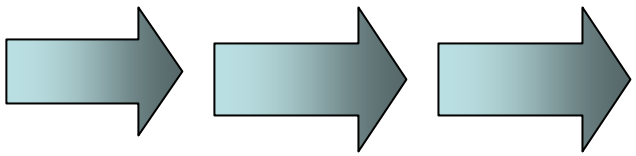
BioReliance®
invitrogen bioservices

BPyV amplification on various cell lines

Cell Line	Copies Passage 1	Copies Passage 5
CHO-K1	10^7	0
Vero	10^7	0
BHK	10^7	0
FRHk	10^7	0
Primary Calf Kidney	10^7	10^5
MRC-5	10^7	0

Rate of cell passage and virus yield

Passage at 3-day intervals



No detectable virus producing cells ($<10^2$)

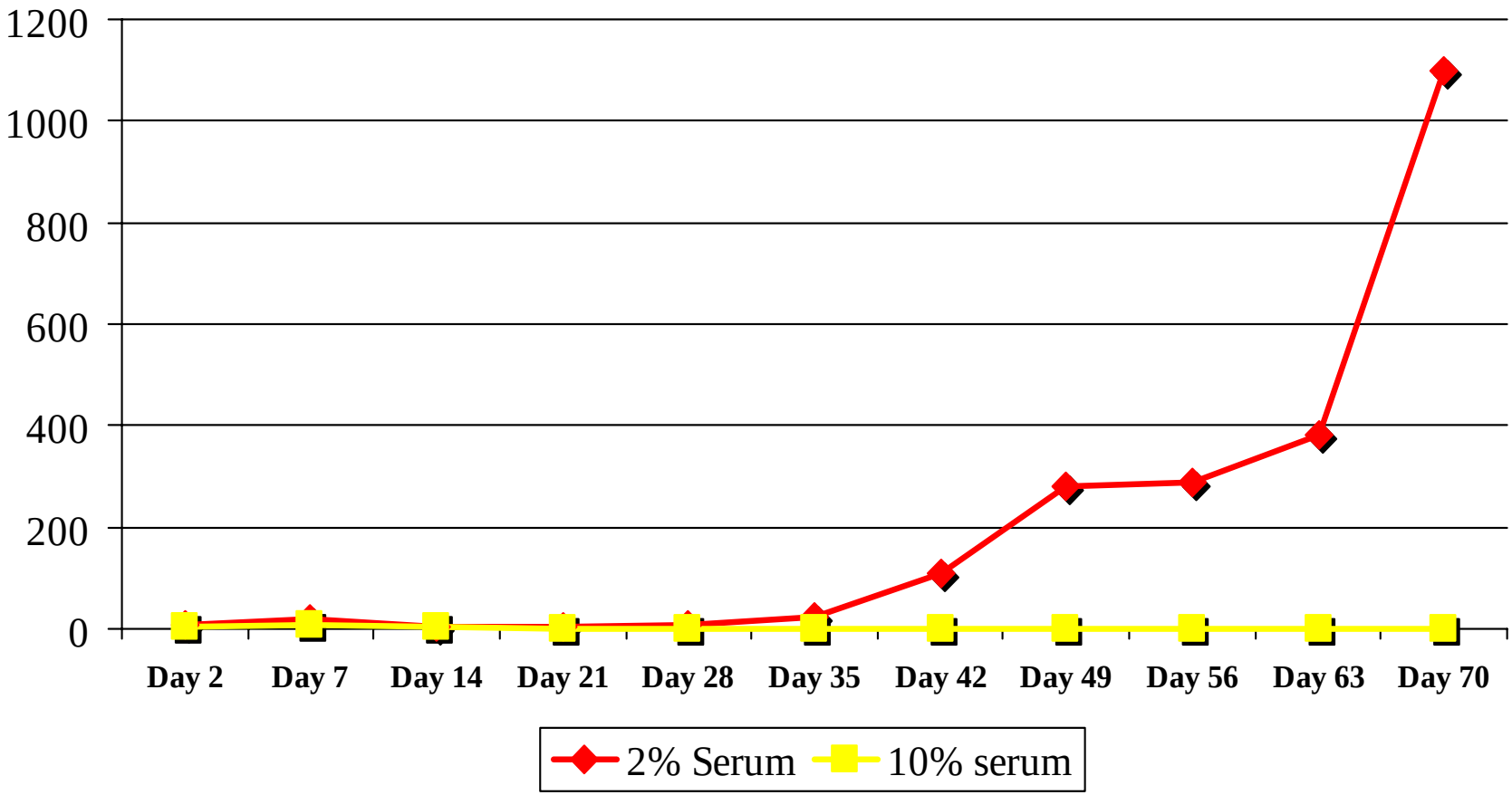
Passage at 2 week intervals

15% of cells virus producing

3 months of passage at 2 week intervals ~ 30% cells virus producing 2×10^4 genomes per cell

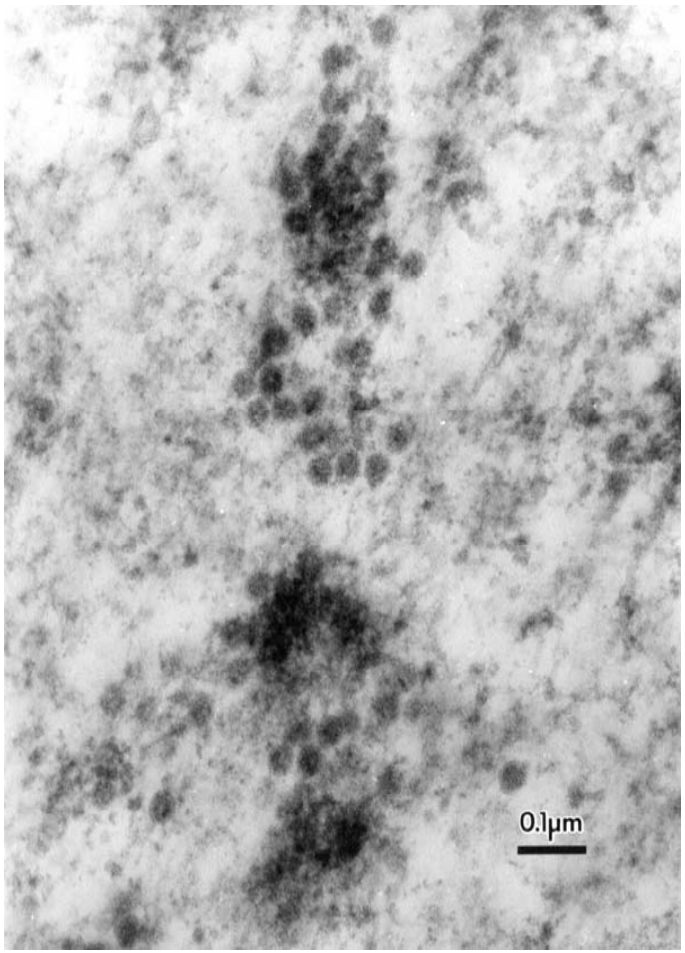
BioReliance
invitrogen bioservices

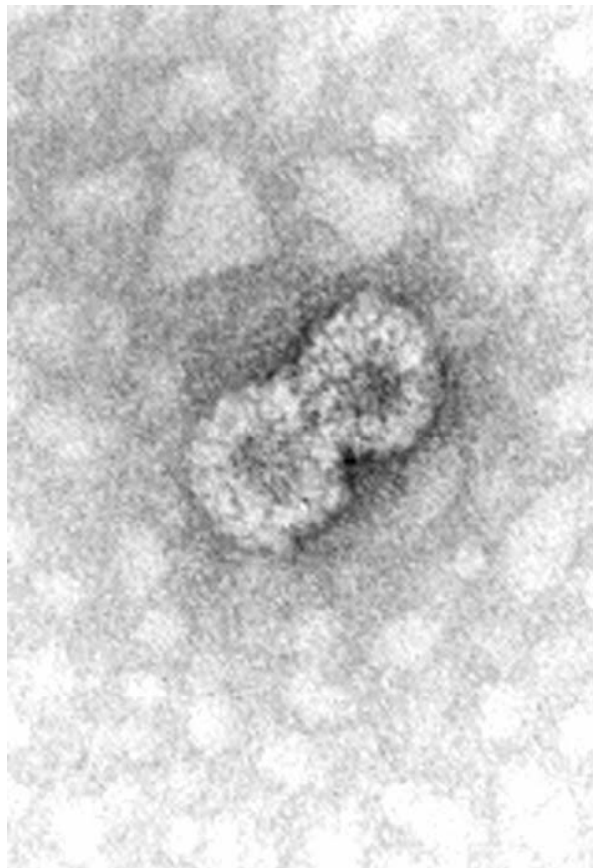
Growth Dynamics of Bovine Polyoma virus



BioReliance®
invitrogen bioservices

BPyV particles

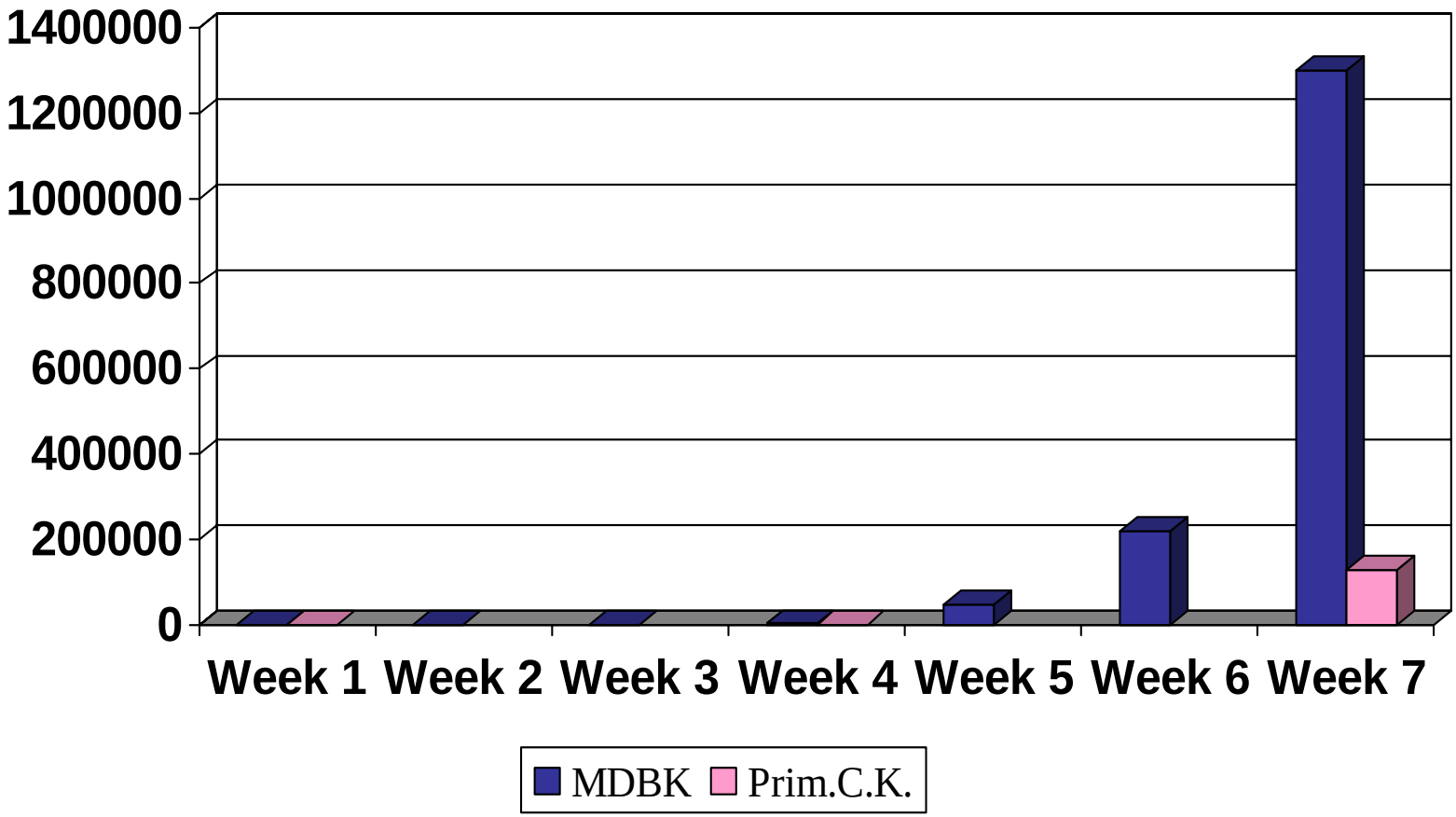




Photographs by Euan Milne

BioReliance
invitrogen bioservices

Amplification of BPyV



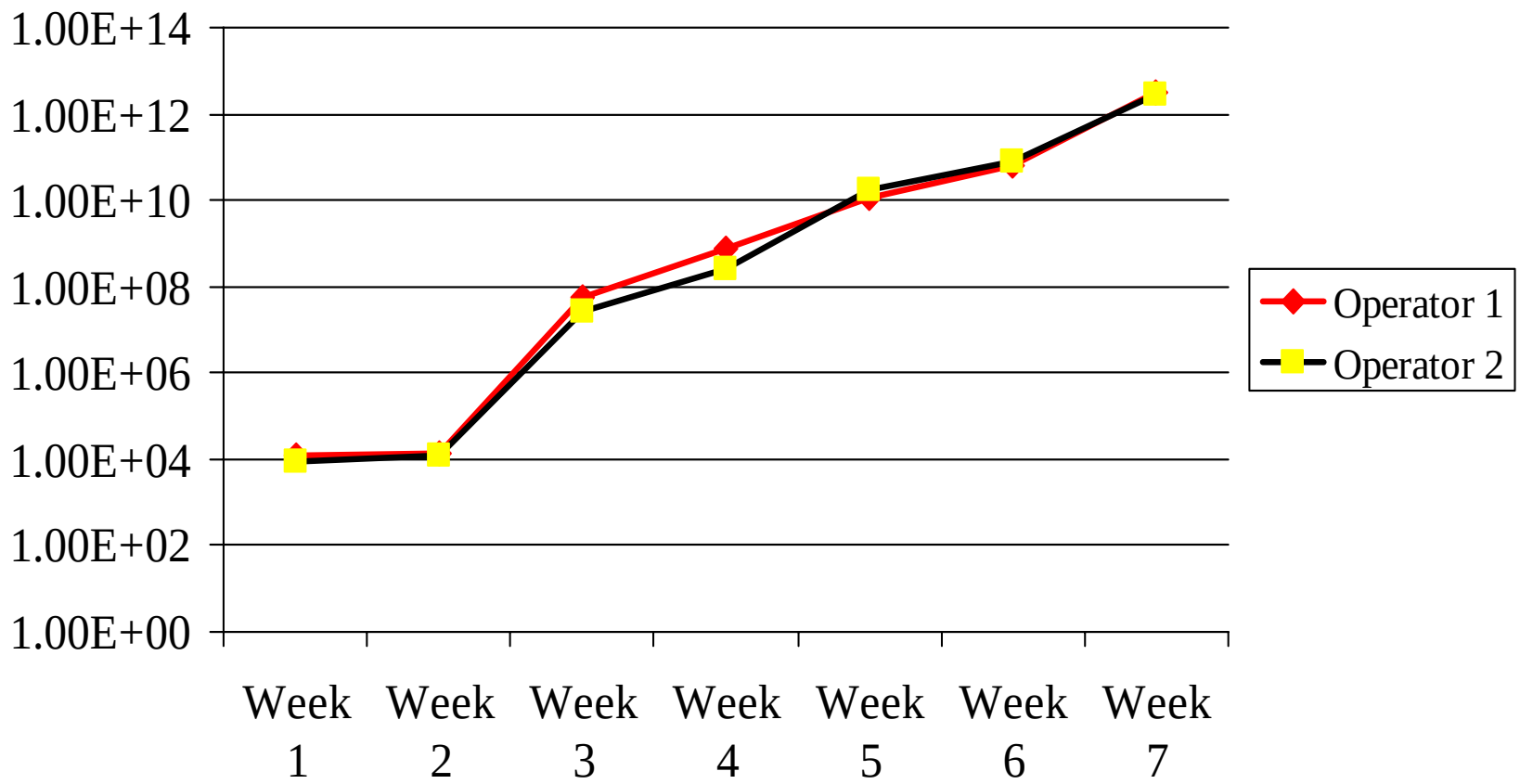
BioReliance
invitrogen bioservices

Validation of MDBK Assay

- Sensitivity and Reproducibility
  - Titration of virus stock from 10^{7} to 10^{-1} carried out in duplicate on MDBK cells by 2 operators- cultured for 7 weeks
- Specificity
  - Virus (10^{7}) inoculated onto MRC-5, CHO-K1, 293 and MA104 cells- cultured for 7 weeks
  - Inactivated virus inoculated on MDBK
- Qualification of Test Material
  - Mock-test performed by inoculating bovine serum spiked with BPyV onto calf kidney cells to determine if virus would be neutralised by serum

BioReliance
invitrogen bioservices

BPyV on MDBK cells



BioReliance
invitrogen bioservices

Testing of Cell Lines by PCR and Infectivity

- BPyV ***not*** detected by conventional PCR in:
  - Human: HEK 293, HeLa, MRC-5, A549, Hs27, RD, MCF
  - Monkey: CV-1, LLC-MK2, Vero
- BPyV ***not*** detected by Q-PCR in:
  - Human: HEK 293, HeLa, MRC-5
  - Monkey: Vero, Cos-7
  - Bovine: BT, MDCK
  - Rodent: CHO, BHK-21, NS0,
  - Avian: QT35
- BPyV ***not*** detected by infectivity assay in:
  - MRC-5, HEK 293
  - CHO

BioReliance®
invitrogen bioservices

Testing Bovine Serum

- 28 batches of FBS tested by both Q-PCR and infectivity assays for BPyV
- All batches of FBS tested by Q-PCR were positive
- Range 1.0×10^3 to 2.6×10^4 genome equivalents/ml
- No BPyV infectivity detected in any batch of FBS tested.

BioReliance®
invitrogen bioservices

Conclusions - Questions?

BVDV

- What is the risk of using high antibody titre bovine serum if the raw material is treated by a validated viral inactivation method?

BPyV

- Is there a significant risk from infectious BPyV in bovine sera?

BioReliance®
invitrogen bioservices

Acknowledgements

- Daniel Galbraith
- Ian Forgie
- Archie Lovatt
- Karen McDonald
- Carron Nairn