VACCINE CELL SUBSTRATE 2004
practical experience

A safety evaluation of MDCK for influenza vaccine production

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Egg-based vaccine manufacture

- **60 year old technology**
- **yields safe, efficacious vaccine**

- **breeding of eggs**
  - *Dependent on flocks vulnerable to diseases*
  - *Order one year in advance*
  - *Controlled quality*

- **egg-based production**
  - *Open system, manual handling*
  - *Variation in starting material*
  - *No advantages for scale-up of production*

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T4

CONTINUOUS CELL LINES - VERO

- **Manufacturing logistics**
  - increased flexibility
  - starting materials in stock
- **Manufacturing economics**
  - limiting yields
- **Manufacturing consistency**
  - consistent starting materials, cell banking
- **Regulatory environment**
  - used for licensed vaccines (polio, rabies)

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Slide 3

T4

Suggestion: place information from slides 3 and 4 side by side under each of the 4 headings you have for both vero vs. MDCK

Delete: slide 5 and merely speak to the advantage of MDCK over vero

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T5

CONTINUOUS CELL LINES - MDCK

- **Manufacturing logistics**
  - increased flexibility
  - starting materials in stock
- **Manufacturing economics**
  - high yields
  - economy of scale
- **Manufacturing consistency**
  - consistent starting materials, cell banking
- **Regulatory environment**
  - unprecedented

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T5 Combine with slide 4

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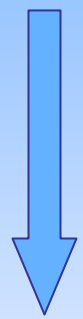
T6

CONTINUOUS CELL LINES

VERO vs. MDCK



prior approval of cell line vs. yield

T6 Delete

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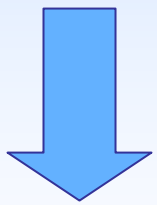
Madin Darby Canine Kidney - 1

- derived from a kidney of a normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby, University of California,
- submitted to American Type Culture Collection (ATCC) August 1964 at p49 by S.H. Madin and N.B. Darby,
- propagated to working cell bank ATCC CCL-34 at p52 directly from the original depositor ampoules and cryopreserved February 1991.

Madin Darby Canine Kidney - 2

- ATCC CCL-34, p52 obtained by Solvay in 1992
- adapted to serum-free conditions in 1992
- banked by BioReliance, Rockville, MD at p56 (MCB) and p57 (WCB) in 1992
- propagated by Solvay to p97 (ECB) in 1992/1993 by methods representative for production



historic track record

CCL - PERCEIVED RISKS

- Tumorigenicity of CCL-intact cells
- (Oncogenicity of) Adventitious viruses
- Oncogenicity of CCL-DNA

CCL - PERCEIVED RISKS

- **Tumorigenicity of CCL-intact cells**
- **(Oncogenicity of) Adventitious viruses**
- **Oncogenicity of CCL-DNA**

TUMORIGENICITY – INTACT CELLS
regulatory guidance

- **ICH Q5D**

  "cells of known tumorigenic potential do not need to be tested further […] for products that do not contain live cells and are highly purified, provided that appropriate limits for residual DNA are met"

- **CPMP Annex NfG on cell-derived influenza vaccines**

- **CBER Points to Consider 1993**

  "human epithelial cells and cells used for live virus vaccine need to be tested for tumorigenicity"

- **CBER**

  "risk ≤10^{-6} per dose acceptable"

  Defined-risks approach to regulatory assessment of use of neoplastic cells for viral vaccine manufacture – CBER draft (Lewis, Krause, Peden)

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TUMORIGENICITY – INTACT CELLS
practical experience

- **Cell line identification (MCB and ECB) –**
  isoenzyme and genetic analysis: canine origin
- **Tumorigenicity testing (ECB) –**
  10^7 viable cells in athymic nude mice: positive
- **Risk-based assessment**

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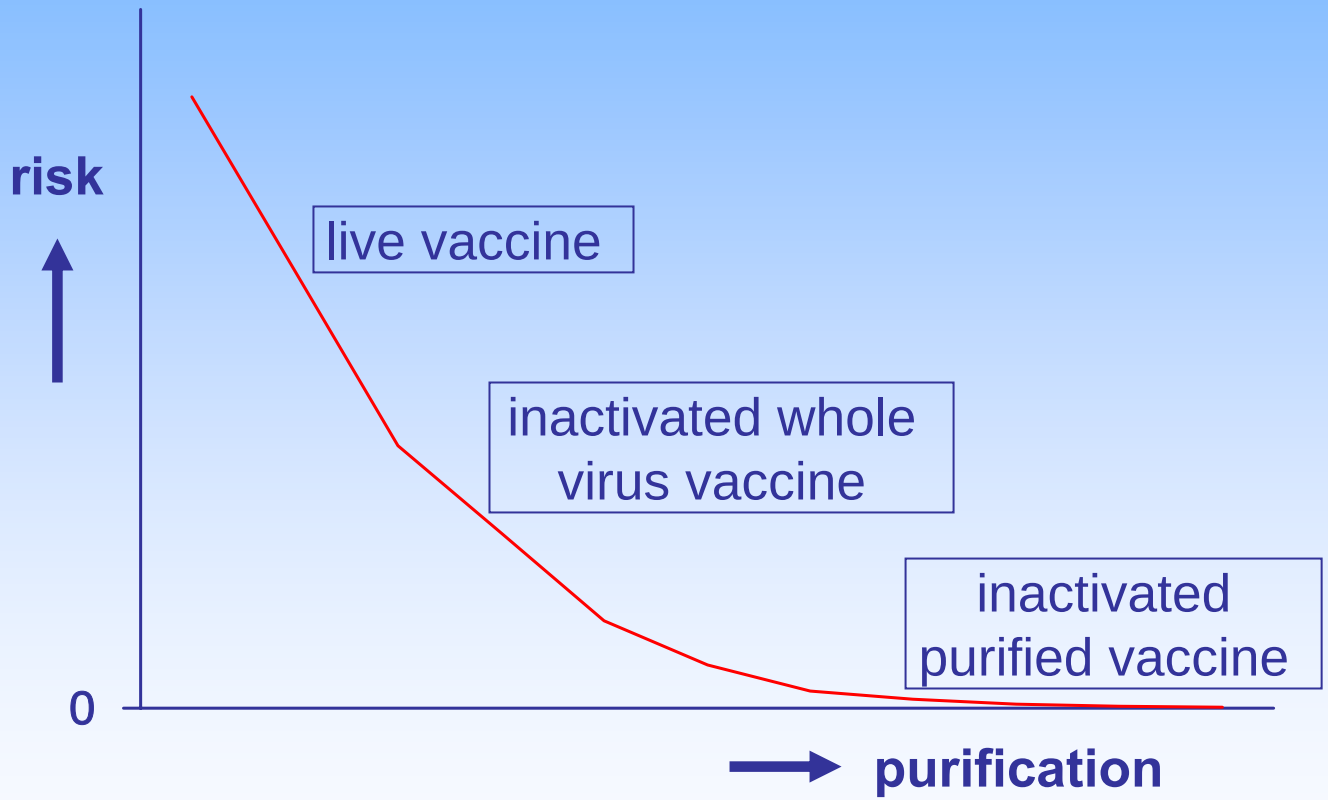
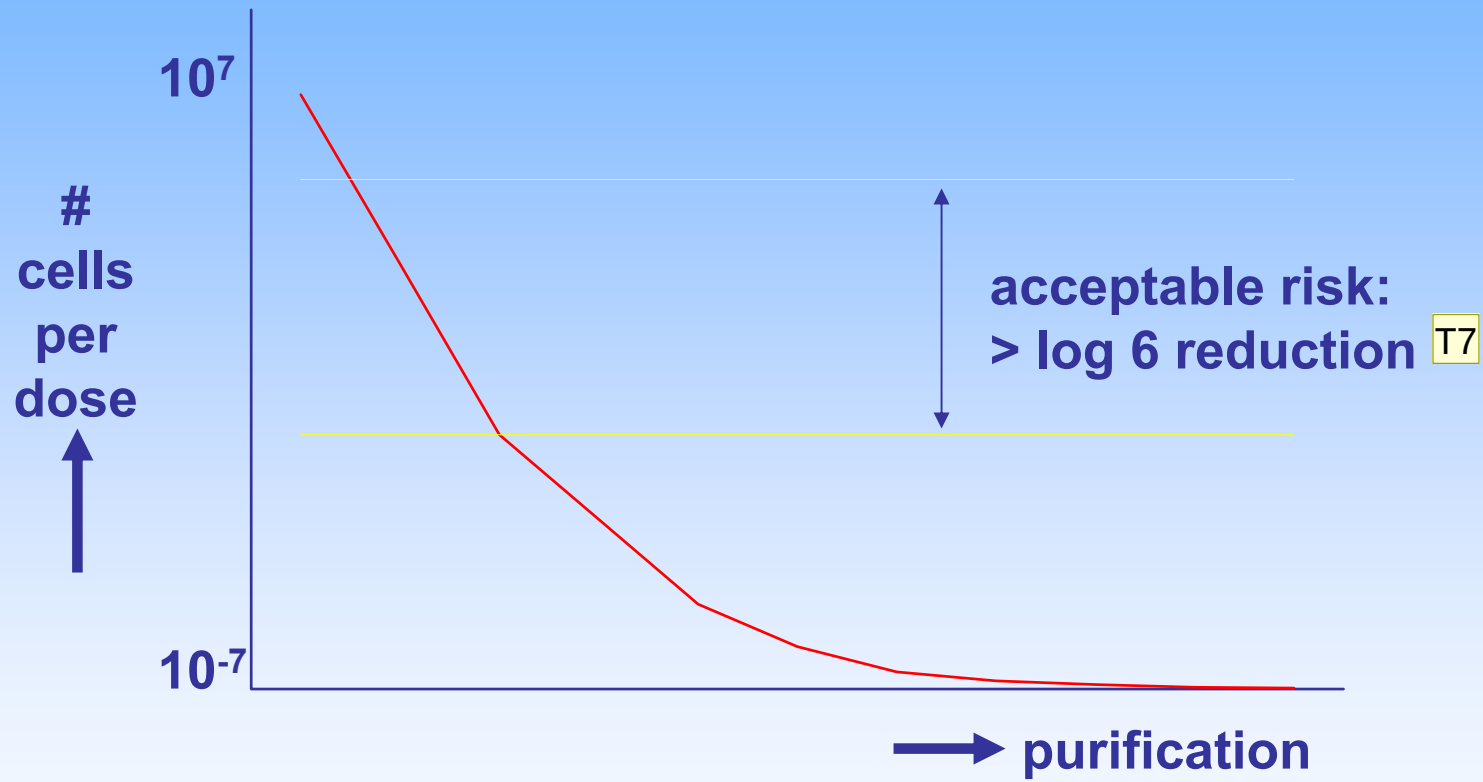
TUMORIGENICITY – INTACT CELLS
practical experience



TUMORIGENICITY – INTACT CELLS
practical experience



- Quantification of tumorigenic potential
- Validated removal intact cells during product purification

T7 or should this read: "> 6 log reduction"?
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CCL - PERCEIVED RISKS

- Tumorigenicity of CCL-intact cells
- **(Oncogenicity of) Adventitious viruses**
- Oncogenicity of CCL-DNA

ADVENTITIOUS VIRUSES
regulatory guidance

- **Absence of adventitious virus testing in cell bank**
- **Validation of removal / inactivation of model viruses by purification process**

ADVENTITIOUS VIRUSES absence testing

- **Aspecific absence of adventitious virus testing (MCB and ECB)**
  - ***in vitro;*** **human, simian and canine cell cultures**
  - ***in vivo;*** **suckling and adult mice, guinea pigs, rabbits and embryonated eggs (allantoic, yolk sac and chorioallantoic)**

- **Specific absence of adventitious virus testing (MCB and ECB)**
  - **Retrovirus testing**
  - **Evaluation of cell bank contaminants (e.g. CAV, CHV, CPV) – CAP test (n=16)**
  - **Identification of MDCK susceptibility – CAP test (n=16)**

no evidence for presence of adventitious viruses

ADVENTITIOUS VIRUSES
validation virus removal/inactivation

- **Model viruses:**
  - **Selection based on relevance (e.g. Influenza)**
  - **dsDNA, (-)ssRNA and (+)ssRNA**
  - **Enveloped and non-enveloped**
  - **Size range 30 – 300 nm**
  - **Low-medium to medium-high resistance**

viral clearance of all model viruses validated

redundant clearance capacity shown

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ADVENTITIOUS VIRUSES
oncogenicity of MDCK lysate

- **ICH Q5D**

  "cells of known tumorigenic potential do not need to be tested further […] for products that do not contain live cells and are highly purified, provided that appropriate limits for residual DNA are met"

- **additional oncogenicity testing in newborn and immunocompromised animals**

lysate of 10^7 cells does not form tumors in 3 months

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CCL - PERCEIVED RISKS

- Tumorigenicity of CCL-intact cells
- (Oncogenicity of) Adventitious viruses
- **Oncogenicity of CCL-DNA**

ONCOGENICITY – CELLULAR DNA
regulatory guidance

- **ICH Q5D**
  "cells of known tumorigenic potential do not need to be tested further […] for products that do not contain live cells and are highly purified, provided that appropriate limits for residual DNA are met"

- **CPMP Annex NfG on cell-derived influenza vaccines**
  "appropriate assay for residual DNA"

- **EP 5.2.3 / WHO**
  "residual DNA < 10 ng/dose for inactivated vaccine"

- **CBER**
  "risk ≤10^{-6} per dose acceptable"

Defined-risks approach to regulatory assessment of use of neoplastic cells for viral vaccine manufacture – CBER draft (Lewis, Krause, Peden)

T2

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T2

give year and name of draft guidance

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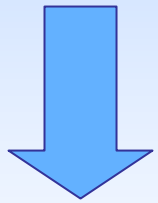
ONCOGENICITY – CELLULAR DNA
practical experience

- **no risks at 100 µg/dose**
- **additional oncogenicity testing with MDCK-DNA (ECB) in newborn and immunocompro-mised animals**



100 µg MDCK-DNA does not form tumors in 3 months

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ONCOGENICITY – CELLULAR DNA
practical experience

- **Residual DNA limit set at 10 ng/dose**
- **Both process validation and lot release testing**
- **Actual values below 1 ng/dose**
- **Actual residual DNA digested to small size**

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Solvay's MDCK cell bank

- **Tumorigenicity of intact cells**
  - only moderate potential in athymic nude mice
  - not in immunocompentent animals
  - risk-based assessment of presence of TP per dose
  - well below acceptable limit, to be confirmed on full production scale

T1

- **(Oncogenicity of) Adventitious viruses**
  - no evidence for presence in cell bank
  - (redundant) clearance of model viruses validated
  - no known risk associated with lysate of 10^7 cells

- **Oncogenicity of MDCK-DNA**
  - no known risk associated with 100 µg
  - residual levels <10 ng/dose, digested fragments

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T1

what does TP stand for?

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MDCK-based vaccine production

cell bank ✓

↓ *cGMP facilities, personnel, materials* ✓

production cell culture *EP 2.6.16 control cells* ✓

extraneous agents testing T9

virus seed ??

↓

bulk virus harvest

↓ *cGMP facilities, personnel, materials* ✓

↓ *validated viral clearance* ✓

final product

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T9

what does TP stand for?

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CC-based influenza virus seed

- **Derived from WHO egg-based vaccine virus**
  - **produced in SPF embryonated eggs**
  - **tested on bacterial and fungal contamination, mycoplasma and ALV**
- **Produced on released MDCK cell bank**

T8

T8

what does TP stand for?

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CC-based influenza virus seed

Adventitious agents testing

- bacterial and fungal contamination, mycoplasma
- *in vitro*: human diploid, simian, canine cell lines
- *in vivo*: suckling and adult mice, guinea pigs
- risk assessment of possible contaminants (co-isolates, susceptibility of eggs and MDCK)

T11

30 PCRs covering 35 human viruses

T11

what does TP stand for?

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MDCK-based vaccine production

cell bank ✓

↓ *cGMP facilities, personnel, materials* ✓

production cell culture *EP 2.6.16 control cells* ✓

extraneous agents testing

T10

virus seed *EP 2.6.16 extraneous* ✓

agents testing + 35 PCRs

bulk virus harvest

↓ *cGMP facilities, personnel, materials* ✓

↓ *validated viral clearance* ✓

\+

final product ✓

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T10

what does TP stand for?

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