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How a New Technology Raised Regulatory Issues and How They Were Resolved: The PERT Assay

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Outline of Talk

- The PERT assays developed
 - Silver-Repaske assay
 - PERT assay
 - Amp-RT assay
- Finding of RT activity in some vaccines
- Initial responses: vaccine safety concerns
- Regulatory perspectives
- CBER approaches to resolving the issue
 - Establish a Working Group (FDA, CDC, Merck)
 - Identify the source of PERT activity in vaccines
 - Investigation of the PERT assay (sensitivity, robustness, etc.)
- Complications with the PERT assay: Assay modifications/improvements
- Resolution and Outcomes



The Reverse Transcriptase Assay: A General Method for Retrovirus Detection

- All retroviruses contain reverse transcriptase (RT)
- RT assays measure the direct incorporation of a labelled deoxyribonucleotide opposite an RNA template
- RTs from retroviruses either use Mg⁺⁺ or Mn⁺⁺ as cofactor
- Assays can generally detect no fewer than 10⁵ - 10⁶ retrovirus particles, *i.e.*, *ca.* 100 to 1000 infectious units
- Assays are not quantitative

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The Product-Enhanced Reverse Transcriptase (PERT) Assay: The Next Generation

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The Product-Enhanced Reverse Transcriptase (PERT) Assay

Three New Methods for Enhancing the Efficiency of Reverse Transcriptase Assay: PCR-based RT assays

- Silver, Maudru, Fujita, and Repaske (NAR 21: 3593-94, 1993)
- Pyra, Böni, and Schüpbach (PNAS 91: 1544-1548, 1994)
- Heneine *et al.* (J. Infect. Diseases 171: 1210-1216, 1995)



Nucleic Acids Research, 1993, Vol. 21, No. 15 3593-3594

An RT-PCR assay for the enzyme activity of reverse transcriptase capable of detecting single virions

Jonathan Silver, Thomas Maudru, Kazunobu Fujita and Roy Repaske
Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Received May 25, 1993; Accepted June 20, 1993

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 1544-1548, February 1994
Medical Sciences

Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement

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Communicated by Harold E. Varmus, November 5, 1993



Journal of Virological Methods 61 (1996) 135-143



Highly sensitive qualitative and quantitative detection of reverse transcriptase activity: optimization, validation, and comparative analysis with other detection systems

Shinji Yamamoto, Thomas M. Folks, Walid Heneine*

Retrovirus Diseases Branch, Division of Acquired Immunodeficiency Syndromes, Sexually Transmitted Diseases, and Tuberculosis Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Mail stop G-15, Atlanta, GA 30333, USA

Accepted 3 May 1996

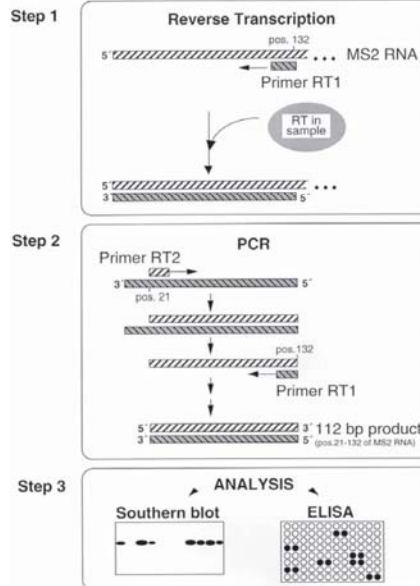
The PERT Assay

Böni and Schüpbach (1996)

PERT assay is approximately 10^6 -fold more sensitive than conventional RT assay

Measures the RT activity of Mn- and Mg-dependent RT

Can detect the RT activity of a single virion



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Licensed Vaccines Were Assessed for Their Reverse Transcriptase Activity by the PERT Assay

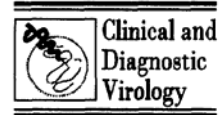
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Certain Live, Attenuated Vaccines Were Found to be PERT Activity Positive



Clinical and Diagnostic Virology 5 (1996) 43–53



Detection of reverse transcriptase activity in live attenuated virus vaccines

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Received 18 September 1995; accepted 17 October 1995

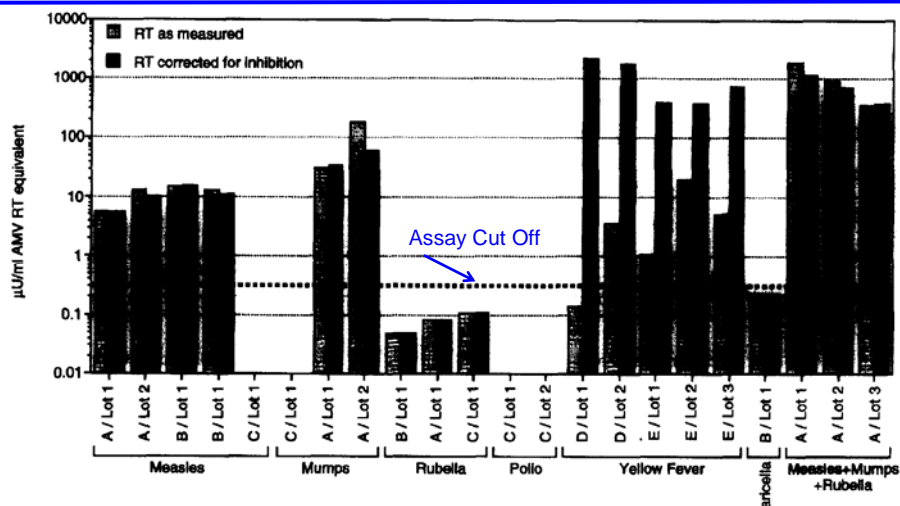


Fig. 1. RT activity levels in live attenuated vaccines determined by the PERT assay. Open bars denote activity levels determined with undiluted specimens; closed bars show activity levels determined by regression analysis from a dilution series of each individual vaccine, as shown in Fig. 2. The broken line indicates the cut-off value of 0.315 μ U/ml.



Initial Responses

- 1996: FDA contacted other national regulatory/control authorities to discuss the issue
- 1996: PERT assay established at CBER
- 1996: FDA convened a meeting with the manufacturer
- 1996: Working Group established (FDA, Merck, CDC)
- 1996: FDA discussed the issues with WHO
- 1996, 1997: FDA convened Vaccines and Related Biological Products Advisory Committee meetings
- 1998: WHO meeting to review data (FDA, NIBSC, Merck, CDC, experts)

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Potential Regulatory Responses to the Presence of RT Activity in MMR Vaccine

- Withdraw the Merck product from the market; use a product manufactured in MRC-5 cells
- Suspend its use until the source of the PERT activity could be identified
- Identify the source of the PERT activity, but keep MMR on the market, as this vaccine had an excellent safety record for over 40 years and no alternative vaccine could supply US needs

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CBER Approaches to Resolving the Issue

- Discussion and cooperation with Merck, NIBSC, CDC, WHO, Paul-Ehrlich-Institut
- Establishment of Working Group
- Investigate the PERT assay to determine its sensitivity/reproducibility/robustness *etc.*
- Identify the source of the PERT activity
- Ascertain whether the PERT activity is associated with an infectious agent

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The PERT Activity in Vaccines Was
Determined to be From The Cell Substrate:
Chick Embryo Fibroblasts

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Possible Sources of the PERT Activity in Chick Embryo Fibroblasts

- Infectious retrovirus that could infect human cells
 - Productive infection
 - Non-productive infection (integration)
- Infectious retrovirus that could infect avian cells but not human cells
- Defective retroviral particles
- Retrotransposons
- Telomerase
- Cellular DNA-dependent DNA polymerases

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Identification of Source of PERT Activity Weissmahr *et al.* J. Virol. 71: 3005, 1997

- Assumed that RT activity arose from a retrovirus
- Used various tRNA primers to generate the cDNA initiated from the primer binding site
- tRNA^{trp} directed cDNA synthesis, but not with tRNA^{pro}, tRNA^{lys1,2} or tRNA^{lys3}
- Sequenced LTRs and used primers to amplify viral genome
- EAV-0_{B1} (endogenous avian virus) was the virus
- EAV is known not to be infectious (Bauer *et al.*, 1976, 1978)

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Infectivity Studies With CEF (cells/supernatants)

- NIBSC (Robertson *et al.* Biologicals 25: 403, 1997)
 - Human cells: MRC-5, WI-38, RD, C8166, CEMx174, THP1, LC5
 - Monkey cells: Vero
 - Rabbit: RK13
 - Turkey embryo fibroblasts
- CBER (Khan *et al.*, J. Clin. Virol. 11: 7, 1998)
 - Human cells: A549, A204, HOS, Raji, PBMC
- CDC (Johnson and Heneine, J. Virol. 75: 3605, 2001)
 - Quail QT35 fibrosarcoma cells

No productive infection could be demonstrated 17

Non-Productive Infection Studies: Can EAV or ALV Particles Enter Human Cells and Integrate?

- A single-round infection of PBMC by the CEF RT particles was assessed using measles virus vaccine equivalent (Merck)
- Infection by EAV and ALV was assessed by PCR
- Integration was evaluated by *Alu* PCR
- No Integration was found

(Shahabuddin, Sears, and Khan, J. Clin. Microbiol. 39: 675, 1998)

PERT Assay: How Sensitive/Robust/Reproducible?

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PERT Assay: How Sensitive/Robust/Reproducible?

- Coded panel of samples prepared at NIBSC and CBER
- Distributed to cooperating laboratories: CBER, NIBSC, CDC, GSK, Merck
- Blinded results compiled at NIBSC and CBER
- Results of CBER panel published (Maudru *et al.*, J. Clin. Virol. (1998)
 - PERT assay is robust
 - Results confirmed Swiss study: RT activity in avian cell-produced vaccines but not in mammalian-cell produced vaccines

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Complications With The PERT Assay

- Because of its high sensitivity, the PERT assay has a background activity
 - Assay background due to Taq DNA polymerase
 - Detects RT activity of several host enzymes
 - Telomerase
 - Retrotransposons
 - DNA-dependent DNA polymerases

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Approaches to Lower False-Positive Signals

- Assay Background Activity
 - Use of thermostable DNA polymerases with reduced RT activities: Pfu, UITma
 - Evaluate the use of various RNA templates: MS2, BMV, TMV
- Cellular Background Activity
 - Identify reagents that lower the cellular background
 - reduced assay pH
 - activated DNA
 - oligonucleotides,
 - heparin

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Adaptation of the PERT Assay to a Quantitative Assay

qPCR technologies TaqMan the PERT assay to a quantitative assay, *e.g.*,

- Q-PERT
- TM-PERT
- F-PERT
- STF-PERT

Assays still are not specific to retroviruses

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Summary

- An assay with increased sensitivity resulted in concerns with a licensed vaccine
- Working groups with all affected parties were rapidly established: FDA, other NRAs/NCAs, CDC, industry, WHO
- NRAs decided that withdrawing the MMR vaccine was not appropriate based on incomplete data and the safety record of the vaccine
- The public was kept informed through open discussions with the Advisory Committee
- Resolution of the issue was through experimental research by the interested parties to demonstrate that the PERT signal was due to non-infectious particles

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Outcomes

- Rapid responses by NRAs, manufacturer, and WHO addressed a potential safety issue with a childhood vaccine
- The PERT assay was determined to be a robust assay; it is now the recommended type of RT assay
- This episode remains a model example of how a potential safety concern raised by the application of a new technology to a licensed vaccine was resolved to the benefit of the public health by the cooperation of all parties and the free exchange of data

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