# Advancing Regulatory Science

# Developing the "Ultimate Tool" to Ensure Live Viral Vaccine Safety

## Viral Mutations Threaten the Safety of Oral Polio Vaccines

- High mutation rate of RNA viruses requires manufacturers of live attenuated RNA virus vaccine to monitor each batch of vaccine for presence of "revertant" viruses with mutations that enable them to cause disease.
- Each batch of oral poliovirus vaccine (OPV) must be tested for neurovirulence in animals and for the presence of revertants; this requires the use of many animals and is expensive
- Previous techniques for detecting unstable sections of RNA virus genetic material by sequencing all genes of RNA viruses have been laborious, of limited sensitivity, or both.
- CBER scientists have been working for over a decade to optimize and improve ways to monitor mutations in live RNA viruses

## CBER Searches for a Better Way to Monitor Consistency and Safety of Live Viral Vaccine Batches

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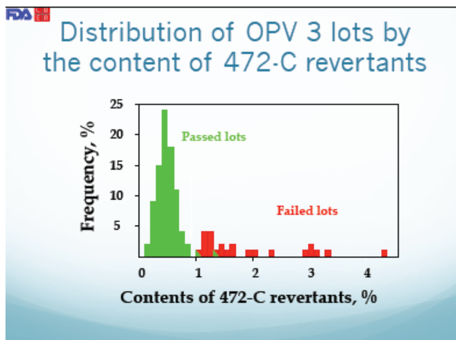
## STEP ONE: MAPREC

(**M**utant **A**nalysis by **P**CR and **R**estriction **E**nzyme **C**leavage)

(*Proc Natl Acad Sci* USA; 1991 88(1) 199-203)

Measures the frequency of neurovirulent mutations at a specific region of the viral genome that correlates with the level of neurovirulence determined by the monkey neurovirulence test



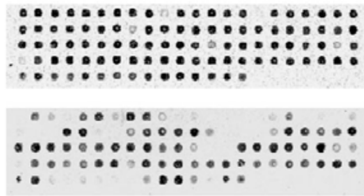

- Quantified the number of revertants with a specific mutation in nucleotide 472, which substitutes the nucleotide cytosine for uracil (472-U -> 472C)
- **Adopted by World Health Organization for ensuring quality of OPV vaccine mutation analysis.**

# STEP TWO:

**MARSH** (Mutant Analysis by PCR and Restriction Enzyme Cleavage)
**MAVR** (Microarrays for Resequencing and Sequence Heterogeneity)

(*Proc Natl Acad Sci* USA; 2003 100(16):9398-9403)

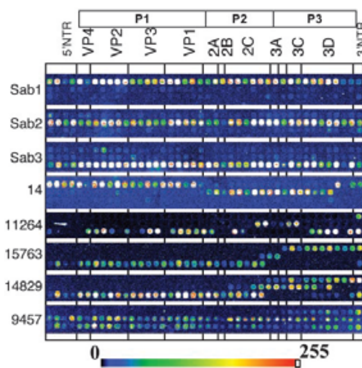
**The combination of MARSH and MAVR produced instant genetic maps of vaccine-derived polioviruses and demonstrated the degree of their evolutionary divergence from the original strain. This suggests that the combination of these techniques might enable large-scale full-genome screening of viral isolates for epidemiological surveillance, vaccine quality control, and analysis to changes in response to drug treatment**



**MARSH analysis of a poliovirus strain (11264) isolated from a person who had contact with a person who had vaccine-associated paralytic poliomyelitis showed emerging point mutations.**

## MARSH

- Identified single nucleotide changes present at low levels
- Identified areas of rapid change and areas of above-average stability in the genome
- Demonstrated that MARSH microchips can be easily produced, making them more practical than industrially produced microchips




**MAVR demonstrated hybridization of Sabin and vaccine-derived polio virus strains (11264, 15763, 14829, 9457). MAVR chips produced a hybridization image that graphically revealed recombination patterns and crossover regions.**

## MAVR

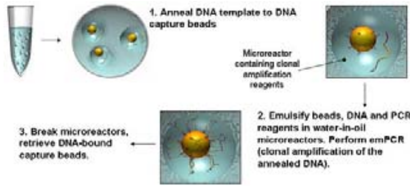
- Showed that strain 14 from a person who contracted vaccine-associated paralytic poliomyelitis was a recombinant of Sabin types 1 and 2
- Showed strain 11264 from a healthy child who had been in contact with the vaccine-associated case was a recombinant of Sabin types 2 and 3
- Suggested that strain 11264 might have regained neurovirulent properties due to its very great divergence from a vaccine progenitor; subsequent testing in mice proved this was true.

# STEP THREE: MASSIVELY PARALLEL SEQUENCING

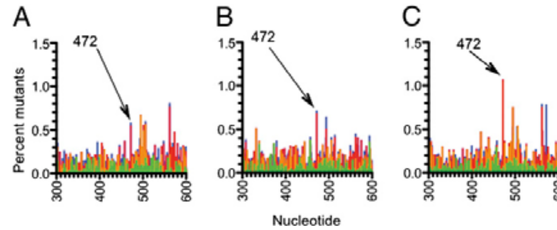
(*Proc Natl Acad Sci* USA; 2010 107(46):20063-20068)

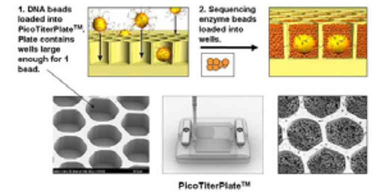
**The type of MPS called "pyrosequencing" uses the so-called "sequencing-by-synthesis" method. This involves sequencing a single strand of genetic material by synthesizing the complementary strand along it. Solutions of A, C, G, and T nucleotides are sequentially washed over the single strand. Each time one of the bases attaches to the growing complementary strand, light is generated, signaling that the specific base in the reaction mixture has bound to an unpaired nucleotide on the original strand.**

## Comparing MPS with MAPREC




Viral gene fragments are attached to tiny beads(1 fragment per bead). Each bead is encased in an oil droplet containing all reactants needed for polymerase chain reaction to amplify the gene fragment. (This illustration uses DNA instead of RNA.)




Encased beads are loaded into tiny reactor wells that contain sequencing enzymes--one bead per well.

**(A)** WHO reference OPV used in MAPREC studies that represents a “passed” batch that has less than 0.7% of the revertant
**(B)** US National Neurovirulence Reference for type 3 OPV (NC2), which represents a marginally acceptable vaccine
**(C)** WHO reference OPV for MAPREC studies representing a “failed” batch that has 1.1% of revertants.

**MPS analysis of validated reference preparations yielded results identical to those previously obtained using MAPREC. This suggests that MPS could replace MAPREC for lot release testing of OPV.**

- MPS identified patterns of mutations near nucleotide 472 in three standardized samples of Sabin type 3 OPV.
- The results suggest that MPS can be used to quantify the proportion of 472-U → 472-C mutants, which determines the level of neurovirulence of the virus stock used to make OPV.

**The CBER scientists reported for the first time that profiles of mutations in vaccine batches can be analyzed by MPS and used to ensure consistency in the manufacturing process—the cornerstone of vaccine safety.**

**THE FUTURE:**

**The work performed for poliovirus may be applicable to evaluating consistency, safety and quality of other biologics.**

**In-house use of MPS provides CBER expertise in this novel technology already being used by manufacturers, thus facilitating familiarity and readiness to evaluate MPS-derived data submitted by sponsors in regulatory submissions.**