ARMWG Titer Analysis

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Statistical Analysis –taking the average of averages

- Calculated values across dilutions then take an average.
  - Locks in statistical sampling variability.
- Pooled the raw data across dilutions then calculate values then take an average.
  - Statistical sampling variability is not as great.
  - These data were not tested data point by data point for the appropriateness of pooling.
- Lab to lab variability not useful.

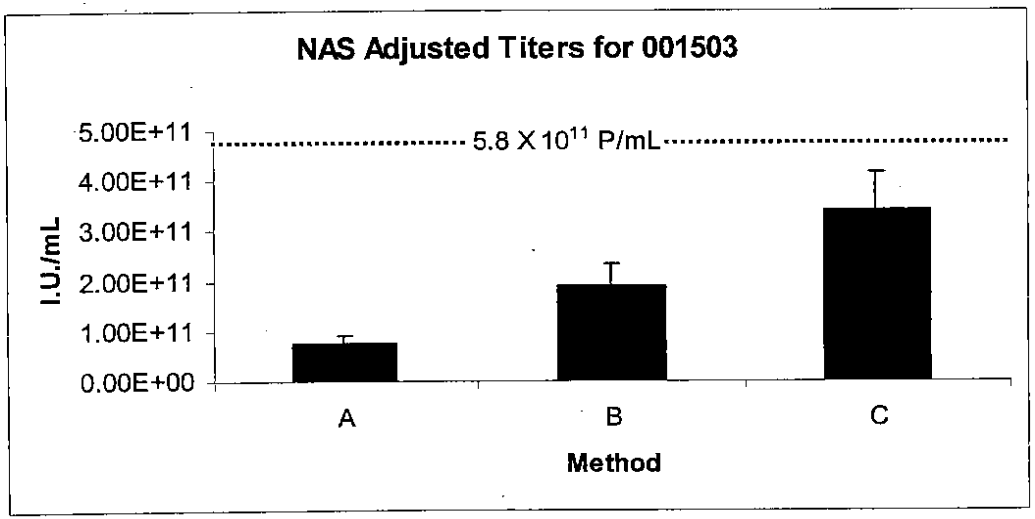
Determined the ARMWG-SOP Infectious Titer-Now What?

- What does infectious titer mean?

"For all animal viruses the number of virus particles in any given preparation exceeds the number of demonstrably infectious units; usually the ratio of infectious units to particles is in the range of 1:10 to 1:1000 or even less. There are two possible explanations for this. The first is that virus preparations contain a majority of noninfectious particles. Although this may be so sometimes, it is unlikely to be the general rule. It is more likely that although all virus particles in a given preparation are capable of causing productive infection, only a small proportion of them are actually successful in doing so. Two lines of evidence support this view. **The first is that the titer of a given virus preparation varies markedly, depending on the nature of the assay system.** (emphasis added)...Therefore, the number of infectious units cannot equal the total number of virus particles. The ratio of the number of infectious units to the total number of virus particles may generally be regarded as a measure of the probability with which virus particles achieve productive infection."

Joklik, W.K. Virology, second edition. East Norwalk, Connecticut, Prentice-Hall, Inc. 1985 p. 11.

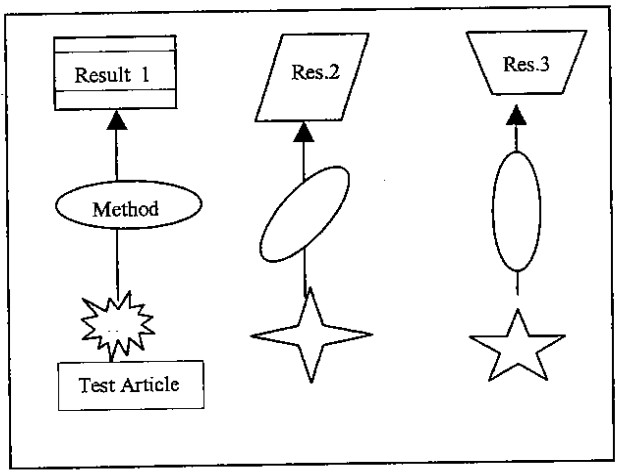
ARMWG 001503 Titer

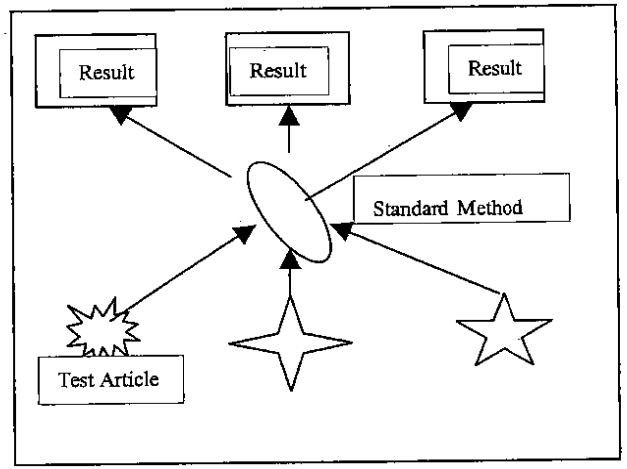


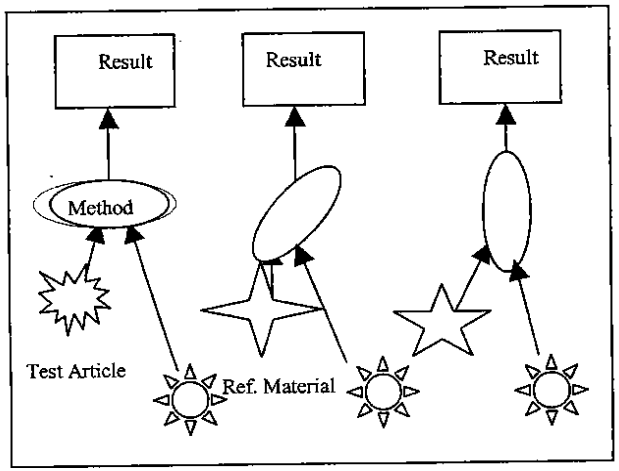
A-ARMWG SOP 80% Confluency
B-ARMWG-SOP 20% Confluency
C-Flow Cytometry Stability Study

- I.U. from different assays are not the same.
- Scaleability from one assay to another needs to be demonstrated.

The Material Is the Reference







Proposal

1. The infectious titer measured by laboratories performing the ARMWG SOP be provided using Jan Callahan's Pooled Estimates. Maximum Likelihood should be recommended as the best estimation method.
2. No standardized spread sheet or other application for calculating titers by any particular statistical method should be distributed or endorsed by the ARMWG.
3. The "label concentration" for the ARM be solely based on the assigned particle concentration 5.8 $X10^{11}$ particles/mL of Infective Adenovirus Serotype 5".
4. A subgroup of ARMWG should be formed, including FDA, to draft guidance for sponsors on how to use the reference material:
   A. For routine testing and for test method validation.
   B. For measuring product specific infectivity.
   C. For determining the absence or concentration of RCA.