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A Hopeful Story of Replacement or Waiving of In Vivo Testing: The Histamine Sensitization Test (HIST) for Final Bulk Acellular Pertussis Vaccines. Juan L. Arciniega, DSc*.

Despite recent setbacks interrupting transmission, pertussis vaccines have substantially prevented severe disease in infants worldwide. Two types of pertussis vaccines are available: whole-cell (wP), containing killed, partially detoxified *Bordetella pertussis* organisms; and acellular (aP), containing one or more purified pertussis proteins. wP vaccines were introduced to industrialized countries in the mid-20th century, but as early as 1981 many replaced wP with aP vaccines to decrease pertussis vaccine reactogenicity. All aP vaccines include pertussis toxin (PTx) chemically inactivated by different means (glutaraldehyde, formaldehyde -alone or in combination- or H₂O₂). Mice are naturally resistant to the effects of histamine but become sensitive upon injection of minute amounts of PTx, allowing for the development of the histamine sensitization test (HIST), which measures residual PTx activity. Although originally designed for wP vaccines, HIST, using either lethal or nonlethal endpoints to measure mouse sensitization, has successfully ensured the release of aP vaccines with acceptable residual PTx activity. Nevertheless, a 2010 international decision to replace HIST with an in vitro test culminated in a 2018 proposal to include the CHO cell clustering assay in the European Pharmacopeia. A personal summary of the roadmap that led to this successful outcome involved:

1. Establishing, by consensus, the test as a priority for replacement
2. Forming a group with a diverse base of knowledge in pertussis to bring about the transition
3. Clearly defining the characteristics of the test to be replaced and of the candidate replacements
4. Holding periodic meetings to regularly review advances
5. Selecting the simplest candidate replacement that performs at least as well as the test it's replacing
6. Considering the possible uses of candidates not selected, instead of dismissing them, while pursuing consolidated steps if more than one protocol for the chosen replacement is available

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