International Validation Study: Phases and Activities (1)

Phase I: Initial Laboratory Qualification

Development of Historical Database for Each Laboratory

- Initial qualification of laboratories by testing agonist and antagonist reference standards and controls
- Establish individual laboratory historical database for standards and controls by conducting independent experiments (10 each for the agonist and antagonist protocols
- Establish initial test acceptance criteria for each lab based on historical database
- Evaluate test method repeatability and reproducibility
- If necessary, modify test method protocols to reduce intra- and/or inter-laboratory variability
- Repeat testing if major protocol changes are required

Phase IIa: Laboratory Qualification/Protocol Optimization

Limited Substance Testing, Possible Protocol Optimization

- At each laboratory, four substances from the ER minimum list of the ICCVAM recommended substances tested independently three times for agonist and antagonist activity
- Evaluate accuracy and reliability
- If necessary, modify test method protocols to reduce variability and/or to improve accuracy
- Repeat testing if major protocol changes are required

International Validation Study: Phases and Activities (2)

Phase IIb: Laboratory Qualification/Protocol Optimization

Additional Substance Testing, Evaluation of Protocol Modifications

- At each laboratory, eight substances from the ER minimum list of the ICCVAM recommended substances tested independently three times for agonist and antagonist activity
- Evaluate accuracy and reliability
- If necessary, modify test method protocols to reduce variability and/or to improve accuracy
- Repeat testing if major protocol changes are required
- Finalize optimized test methods for use in Phases III and IV

Phase III: Laboratory Validation Testing Phase

- At each laboratory, the remaining 41 substances from the ER minimum list of the ICCVAM recommended substances are tested once for agonist and antagonist activity using the final optimized protocols
- Data used to evaluate overall test method performance

Phase IV: Expansion of Validation Database Using Additional Reference Substances

- In the lead laboratory, the 25 remaining substances from the ICCVAM recommended substances are tested once for agonism and antagonism activity using the final optimized protocols
- Data used to further characterize test method accuracy