Development of Quality Assurance Recommendations for the *C. dubia* Toxicity Test

Project Scope and Workplan

Alvina Mehinto

Head of Toxicology Department

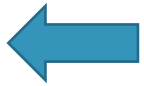
Road Map for this Agenda Item

- Provide overview of scope of work
- Discuss study workplan
- Review schedule and key milestones

Scope of work overview

Study goal: *Develop best practices guidance to improve confidence in the use and interpretation of C. dubia test results*

- Develop shared governance
  - Stakeholder Committee and Science Panel
- Evaluate and optimize test method parameters
  - Develop and execute study workplan
- Produce guidance document



<u>Goal for today</u>

Review and refine the concept of the study workplan

Study Approach

Task 1- Identify potential sources of variability within and among laboratories

- Analyses of laboratory methods and historical data
- **Analyses of split samples by state-accredited labs**

Task 2- Optimize test method and QA parameters to minimize variability

- Targeted studies by 1 or 2 expert labs
- Draft recommendations to reduce variability

Task 3- Evaluate efficacy of test method and QA refinements

- **Interlaboratory comparison exercise with state-accredited labs**

Developing Study Workplan

Initial emphasis on Task 1

- Outcomes and external funding will determine how to conduct tasks 2 and 3

Oral presentation to collect feedback from

- Stakeholders
- Science Panel

Written workplan

- Stakeholder review
- Final approval by Science Panel

Task 1- Options to identify sources of variability

- Analyses of laboratory methods and historical data
  - Extract variable factors from SOPs, QAPs, supporting documents
  - Collate historical data from participating laboratories
  - Send questionnaire and/or one-on-one interviews to collect details on implementation of lab protocols

- Laboratory analyses of split samples
  - Blind analyses of created samples (e.g., dilution water)

Lab method variable examples

Dilution water

- Recipe
- Supplies vendor
- Source water (e.g. DI, Double DI, Milli-Q, etc..)
- Shelf-time
- Water quality data (hardness, pH)

Food

- YCT recipe/ vendor
- Algal culture origin
- Algal culture media
- Shelf-time

Culturing

- Origin of brood stock
- Frequency of restart/turnover
- Frequency of culture failure
- Culture sheet observations

Historical testing data

Focus on Controls

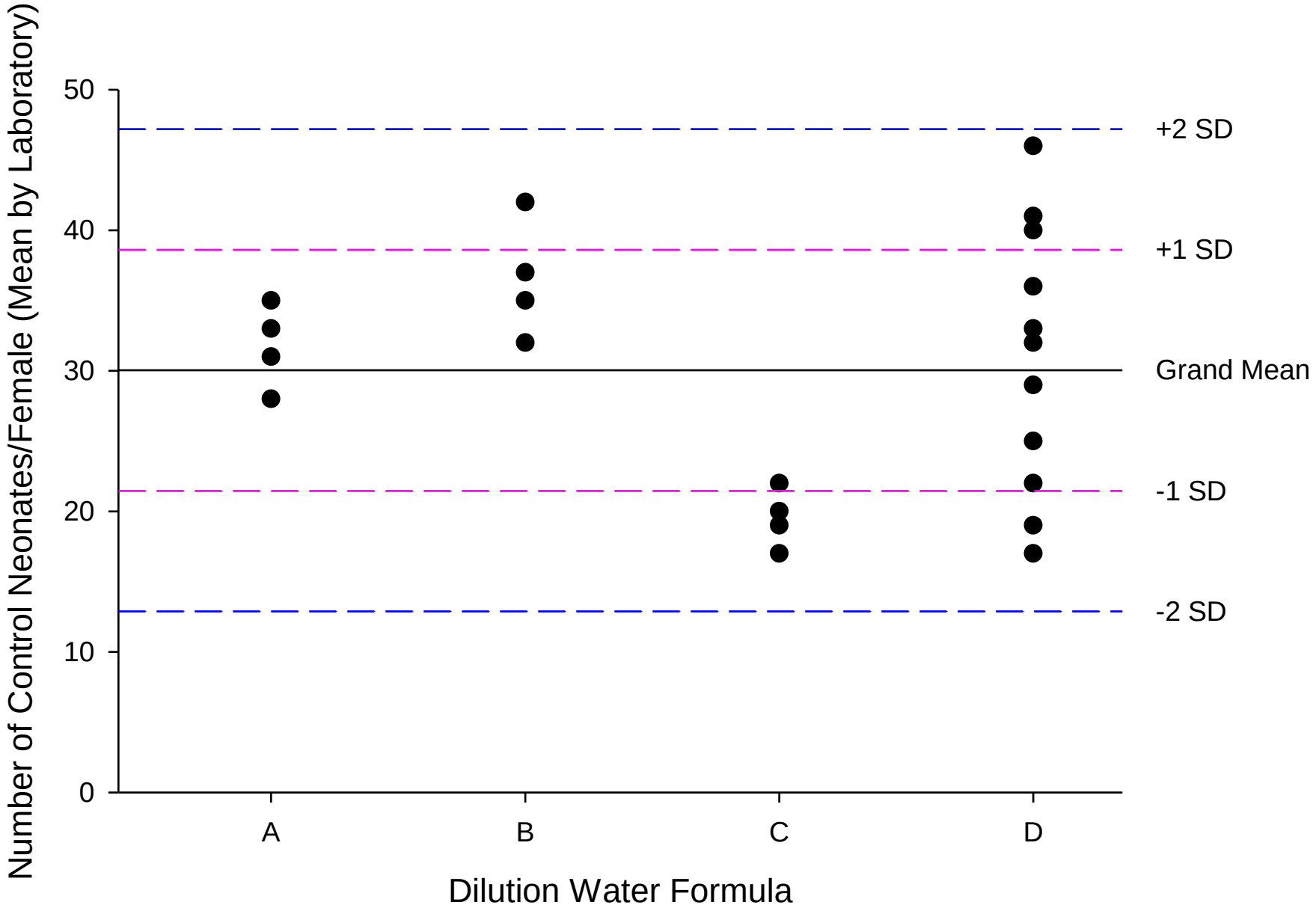
- # of neonates/female
- # of broods/female
- Daily neonate counts
- Time to reproduction
- Frequency of test failures
- Water quality parameters

Data collection methods

	SOP/QAPP/ supporting doc	CETIS/ Data report	Questionnaire
Dilution water			
Recipe	x - SOP		
Supplies vendor	x - SOP		
Source water	x - SOP		
Shelf-time			x
Culture water quality data	x - Support. doc		x
Food			
YCT recipe , vendor	x - SOP		
Shelf-time			x
Algal species, source, culture media	x - SOP		
C. dubia culture			
Origin of brood stock			x
Frequency of restart/turnover	x - QAPP		x
Frequency of culture failure			x
Culture sheet observations	x - Support. doc		
Historical data			
Control variability		x	
# of neonates/female		x	
# of broods/female		x	
Daily number of neonates		x	
Procedure to exclude 4th broods	x - QAPP		
Time to reproduction			x
Frequency of test failures		x	x
Test water quality data		x	x
Experience			
Training logs	x - Support. doc		x
Technical experience			x

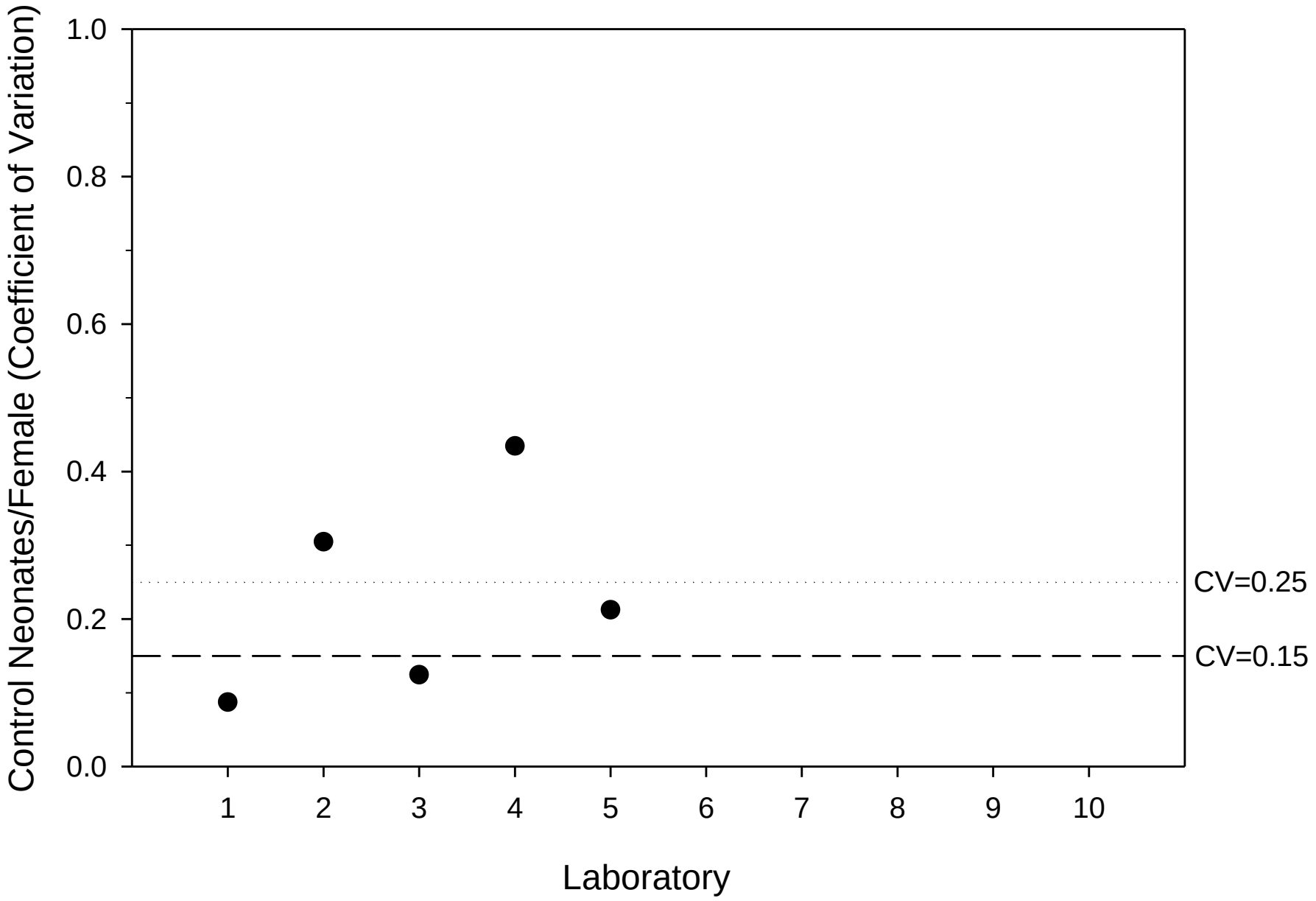
Example : Identifying Variability Across Dilution Water

- Data not real!
- Each dot is a different lab



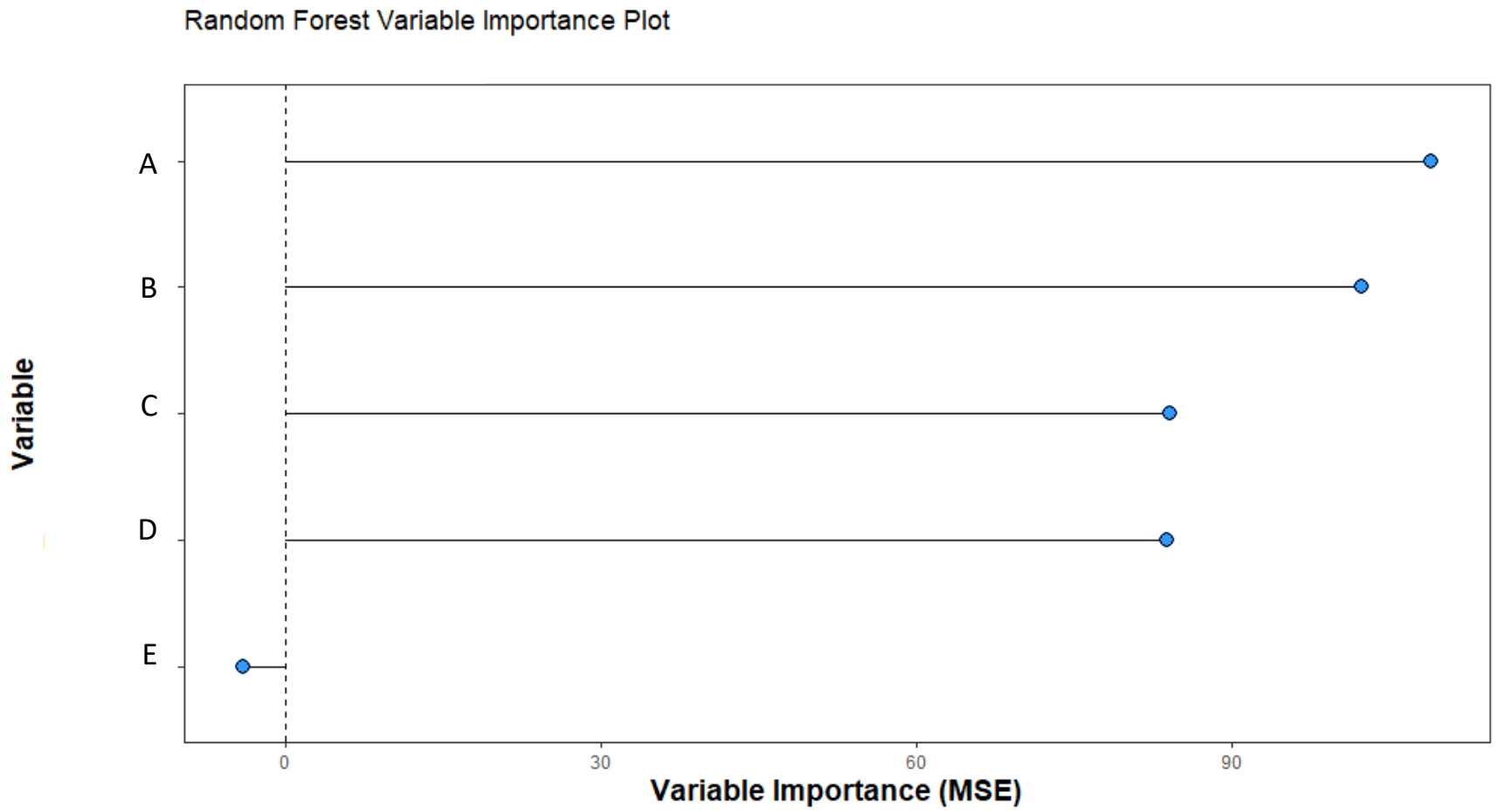
Example: Identifying Variability Across Labs

- Data not real!
- Control CV incorporates both Mean and SD



Example : Identifying Variability Across Multiple Parameters

- Data not real!
- Multivariate analysis to quantify greatest sources of variability

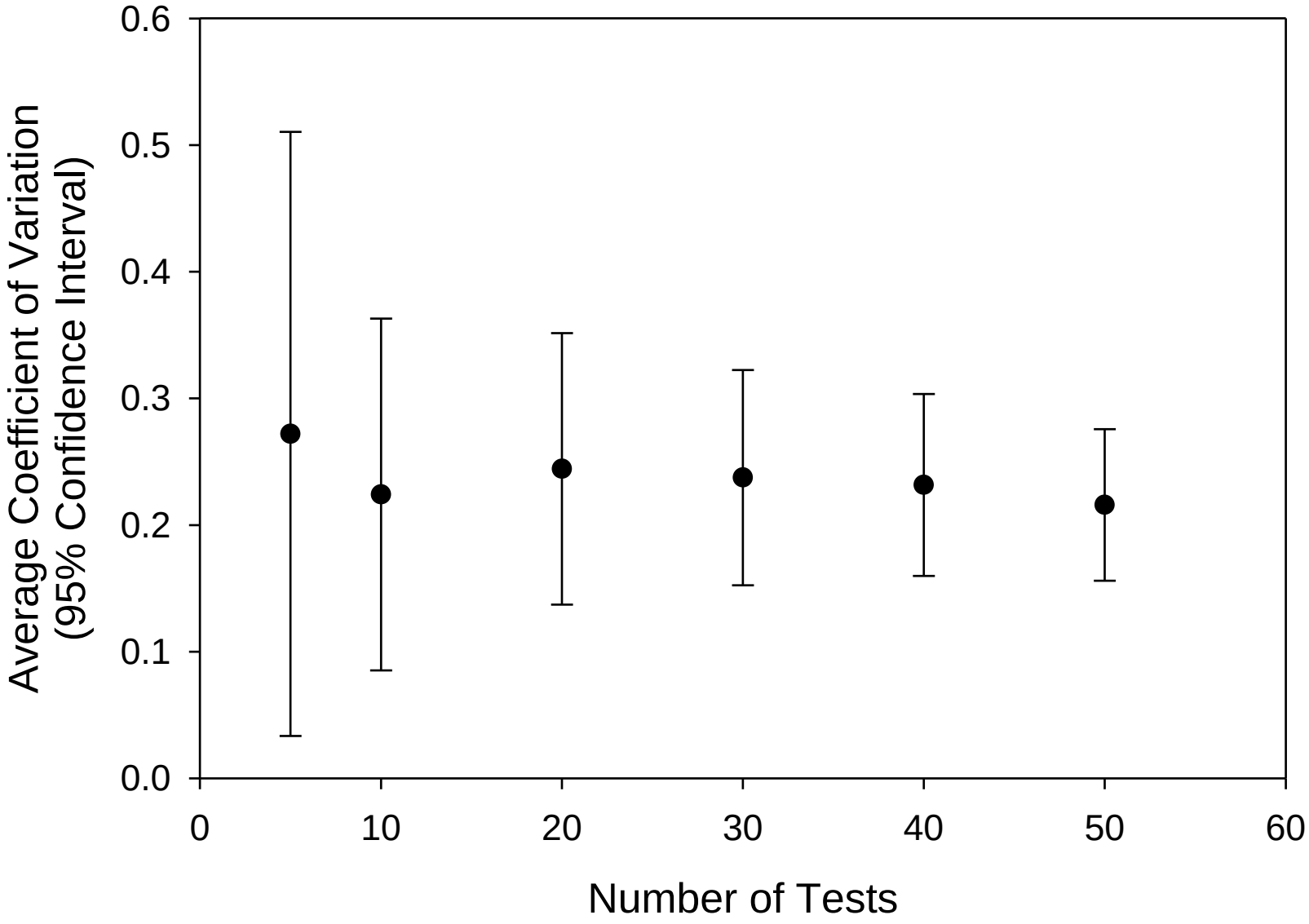


Data collection effort

- What is the right number of tests?
  - How long a time span?
- Too few tests and potentially inflate variability
  - Simple function of sample size vs confidence
- Too long a time period and potentially introduce variability
  - Personnel training, turnover
- We are recommending 30 tests or 3 years

Examining Confidence in Coefficient of Variation (CV)

- Simulated controls between 15-30 neonates/replicate
- Analyses repeated at sample sizes ranging from 5 to 50



Split sample analysis

- Sample selection should be specific to factors we want to evaluate
  - E.g. dilution waters with varying hardness
- Specific factors will likely become apparent after the lab information and historical data analysis
- But detailed approach can only be developed once we know what additional resources are available for this project

Schedule

- Study Workplan
  - Draft by 3/1/21
  - Final workplan by 5/1/21
- Task 1 deadlines
  - Lab data analyses by 7/1/21
  - Split samples analyses by 1/1/22
- Task 2 optimization by 3/1/22
- Task 3 interlab comparisons
  - By 7/31/22
- Final report
  - Draft by 11/1/22
  - Final by 12/31/22

Questions?