C_T values: method validation and decision making
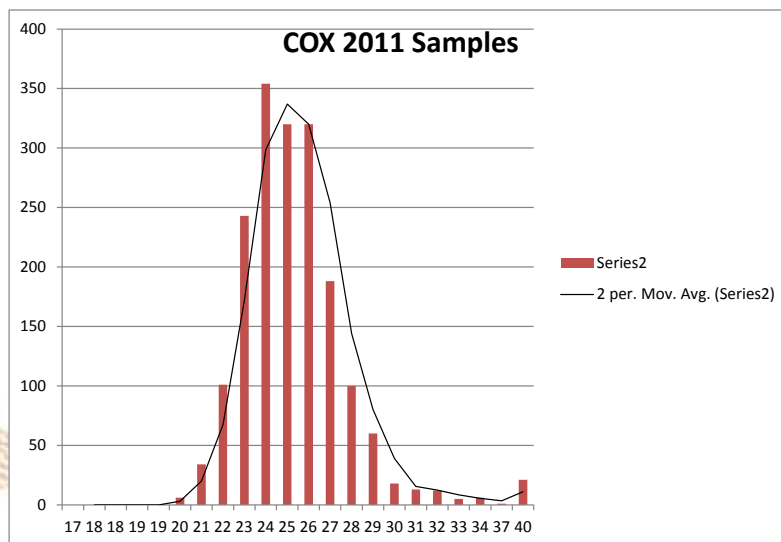
Rebecca Weekes, UK

Overview

- Approaches used at fera
- Example – COX
- Example – *P. ramorum*
- Example – characterising limit of detection
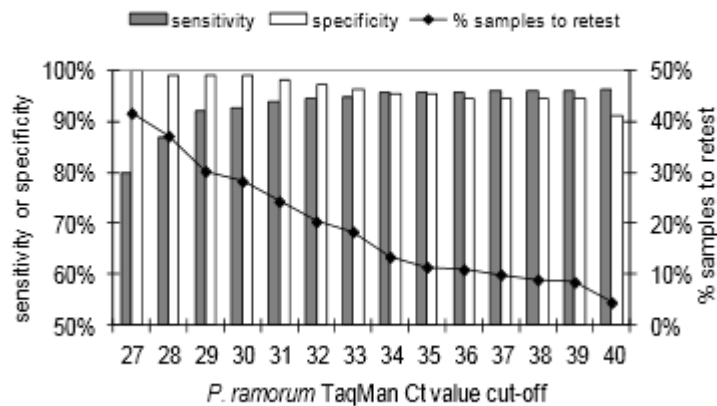- Diagnostic decisions

Approaches used at fera

- Mixed!!
- Case by case
- Pragmatic -
- R&D v Routine Diagnostic
- "a line in the sand which prompts a question and another experiment …."

Distribution of C_T values

- Collecting data from validation study and diagnostic tests.

- Use this data as an indicator of expected performance.
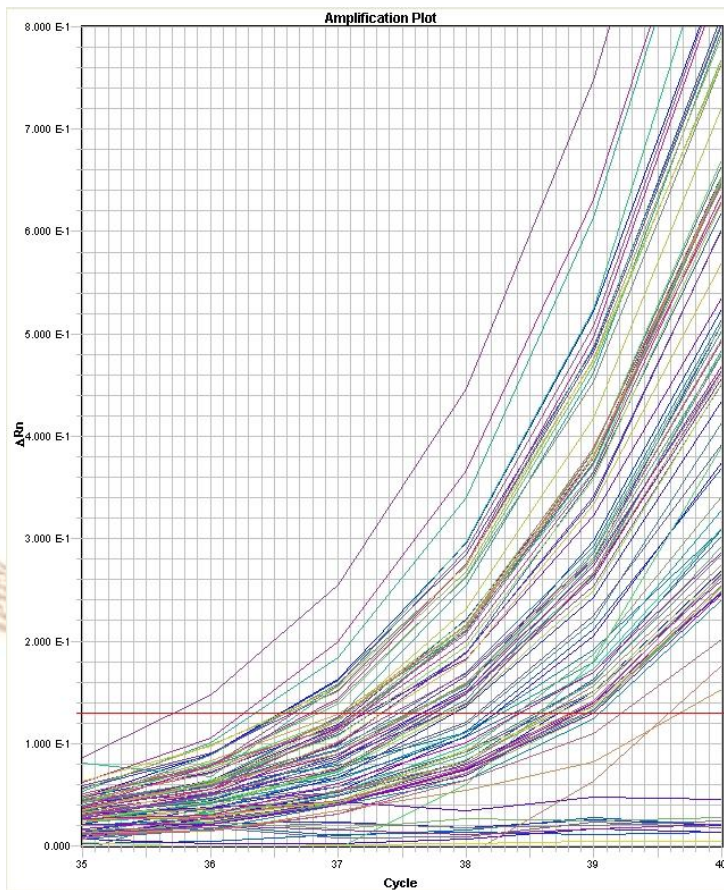
P. ramorum: comparing effect of cut-off values

- The effect of using different cut-offs on sensitivity and specificity.
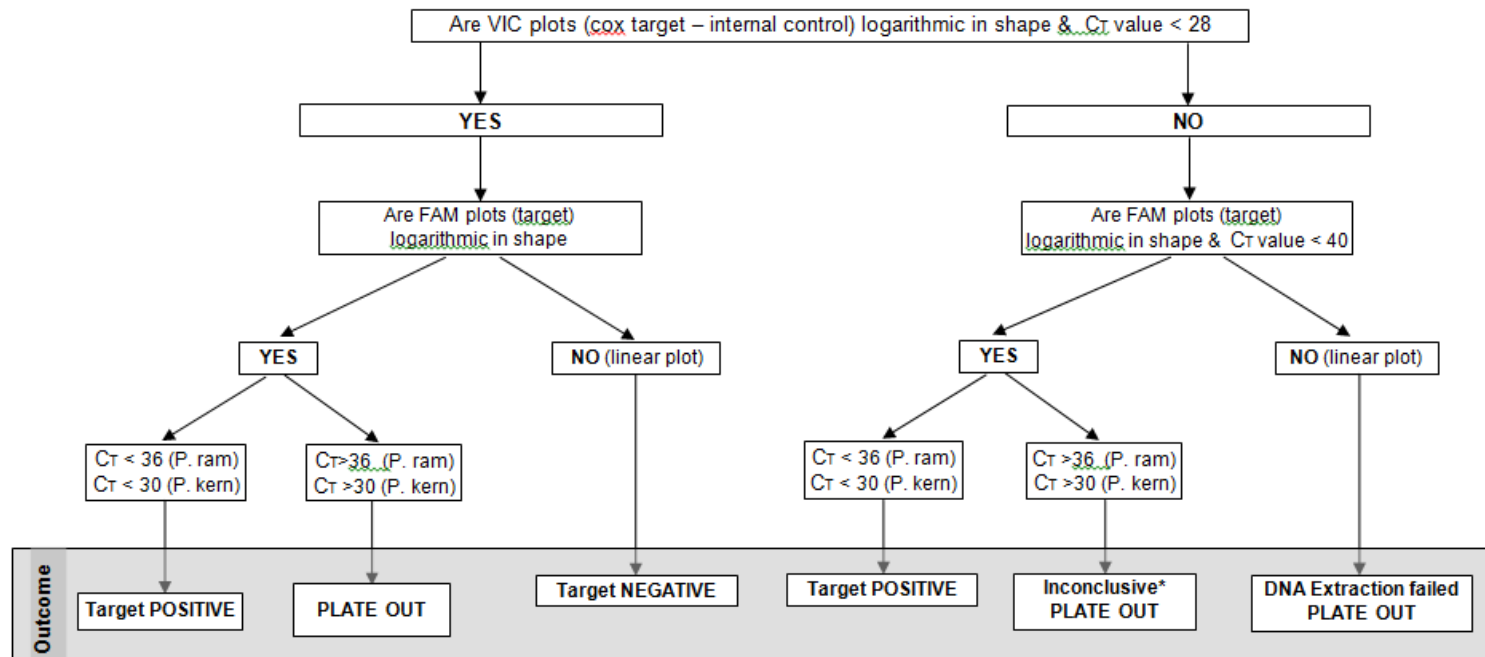
Characterising limit of detection

- Running multiple samples at the limit of detection.

Decision making

Decision Tree For Analysis of *P. ramorum* and *P. kernoviae* TaqMan Analysis.

Are VIC plots (cox target – internal control) logarithmic in shape & C_T value < 28

YES	NO

YES branch:
Are FAM plots (target) logarithmic in shape

- **YES**
  - C_T < 36 (P. ram) / C_T < 30 (P. kern) → **Target POSITIVE**
  - C_T >36 (P. ram) / C_T >30 (P. kern) → **PLATE OUT**
- **NO (linear plot)** → **Target NEGATIVE**

NO branch:
Are FAM plots (target) logarithmic in shape & C_T value < 40

- **YES**
  - C_T < 36 (P. ram) / C_T < 30 (P. kern) → **Target POSITIVE**
  - C_T >36 (P. ram) / C_T >30 (P. kern) → **Inconclusive* PLATE OUT**
- **NO (linear plot)** → **DNA Extraction failed PLATE OUT**

Outcome

*Report as 'Possibly infected, but could not be confirmed. Plate out'

Overview

- There are no hard and fast rules
- "fit for purpose" is essential
- Impact of false positive/negatives
- Bring together experimental approaches and how best to interpret their outcomes.