Josée Laframboise - DFO Lab Discussion

From: Timothy Davis
To: Klotins, Kim; Pedersen, Victoria
Date: 10/20/2011 11:29 AM
Subject: DFO Lab Discussion
Attachments: PCR CHECKLIST.doc

Highlights of yesterdays discussion with Nellie

PCR ISSUES

ZONATION

- Tissue Preparation area - separate room
- Designated areas/benches- cleaned between batches
- Reagent room- no DNA/RNA in this room
- Amplification Zone- no equipment leaves this room
- Secure access to all separate rooms
- Potential of cross-contamination from students

RECEPTION OF SAMPLE

- Was tissue frozen?
- Was RNAlater used? – RNAlater after freezing- useless
- Tissue frozen at -20° C- RNA degredation- Important to have 2ndry assay
- Quantity of RNA received?- quantified on spectrophotometer
- This doesn't give QUALITY of RNA- ie. Degraded due to freezing may not give the required sequence for amplification.

PCR

- Extraction- QA/QC- add water blank. Various methods. 1 every 15
- Reverse transcription to cDNA- Important to have water blank and RNA positive control
- qPCR- Add cDNA positive control- usually in last well to prevent cross contamination. Positive control is artificial- distinct from true positive
- Different pipettes to be used
- Detection was at ~30 cycles- indicates medium to low viral load
- Control detected at 17 cycles- possibly indicates that plasmids are used as control. Problem in using plasmids is that you must be very careful to prevent bacterial overgrowth and gross contamination of whole lab. Cycle 17 would indicate that a very high plasmid load was used as a control- cross contamination risk.

ISSUES

- No tissue to perform second confirmatory test
- No sequence of assay performed to give match of isolate
- References to research paper- does not explain testing method.