Validation Overview for Quantitative Polymerase Chain Reaction (qPCR) Markers

1. SCOPE AND APPLICATION

1.1. As new qPCR markers are added to expand the laboratory's capabilities, they are validated to ensure reliable results. This SOP outlines the steps required to select and test a new marker, and to incorporate it into the lab's analyses.

2. CHOOSE MARKER AND ORDER SUPPLIES

2.1. Determine which marker(s) are to be tested. The markers should detect fecal contamination in surface waters from animals found in Florida. The markers should have adequate sensitivity and specificity (>0.8 for both) and preferably should have been tested by multiple groups and have several literature citations. A license to use patented markers must be obtained if applicable.

2.2. Order necessary reagents (see DEP SOP PCR-5.3 for details).

3. DETERMINE OPTIMAL RUNNING CONDITIONS

3.1. Prepare primers, probe, and standards. Test various concentrations to determine optimal standard curve and running conditions (see DEP SOP PCR-5.4 for details).

4. DETERMINE SENSITIVITY AND SPECIFICITY

4.1. Test positive and negative fecal and water samples prepared from both purified and crude extracts. Calculate sensitivity and specificity (see DEP SOP PCR-5.5 for details).

4.2. Compare calculated sensitivity and specificity to published results. Determine if marker should be added to toolbox and under what conditions it should be run. For example, a marker might only be used with purified samples, for coastal water bodies, near cattle farms, etc. If marker will be added to lab analyses, continue to step 5.

5. CALCULATE LOD AND LOQ

5.1. Calculate limit of detection (LOD) and limit of quantification (LOQ) (See DEP SOP PCR-5.6 for details).

5.2. Update LIMS: add Test ID, limits, spikes, standards. Test calculations and compare to manual calculations.

5.3. Update lab files: reaction plate worksheet, StepOnePlus input file, plate layout worksheets

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6. REFERENCES

6.1. DEP SOP PCR-5.3: Ordering supplies for qPCR markers

6.2. DEP SOP PCR-5.4: Determining optimal running conditions for qPCR markers

6.3. DEP SOP PCR-5.5: Determining sensitivity and sensitivity of qPCR markers

6.4. DEP SOP PCR-5.6: Calculating limit of detection (LOD) and limit of quantification (LOQ) for qPCR markers

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