



Department of Health

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Streamlined Guidelines and Submission Template for the Rapid Validation and Approval of Molecular Assays for Orthopox and/or Monkeypox Virus, June 2022

Please submit all information as outlined below to: clepval@health.ny.gov.

Note that all submissions will be categorized as High Risk and testing may not commence until explicit approval is granted. A Risk Attestation Form is not required with this submission.

All materials are maintained under strict confidentiality. As relates to New York State's Freedom of Information Law (commonly called FOIL): the Department's Records Access Officer has advised Wadsworth Center that if documents are marked "proprietary" or "confidential" or with any labeling indicative of the submitter's desire for an increased level of protection based on the submission content, such protection from immediate release based on a FOIL request is justified. Laboratories will be given an opportunity to block information release if a request for the material is filed under the FOIL by presenting evidence that the materials contain trade secrets. Marking should minimally appear on the cover page of each unit of material. Documents not marked with such terms will not block release of the submission through a FOIL request.

Section 1: GENERAL INFORMATION

Laboratory Name:

NYS PFI #:

Contact Person:

Phone:

Fax:

Contact E-mail:

Assay (Ortho or Monkeypox):

Target Population:

Methodology (chemistry/platform):

Target genes (including internal controls):

Validated Specimen Type(s):

Clinical Purpose (detection, quantification):

Laboratory Director/Assistant Director (NYS CQ Holder for Virology)

CQ Code

Signature

Laboratory Director (if not the responsible CQ Holder for Virology)

CQ Code

Signature

SECTION 2: INSTRUCTIONS FOR SUBMITTING A FULL VALIDATION PACKAGE

These instructions apply to Laboratory Developed Tests (LDTs) that utilize Analyte Specific Reagents, kits labeled for Investigational Use Only (IUO) or Research Use Only (RUO), or assays for which the reagents (oligos) have been designed by the submitting laboratory.

The submitted package must be organized as numbered or tabbed attachments with all pages individually numbered.

Please provide the following:

1. Overview
 - a. Brief explanation of the assay.
 - b. Target gene or region with location of primers and probes.
 - c. Oligonucleotide list with sequences.
 - d. Target population.
2. Standard Operating Procedure, including:
 - a. Detailed step-by-step protocol. This protocol should include specific biosafety practices that will be utilized in the laboratory. BSL-3 practices should be used within a BSL-2 facility when work with infectious material is being performed. All work with potentially infectious material should be performed within a certified Class II biosafety cabinet.
 - b. Specimen collection, processing, and storage requirements.
 - c. Acceptable specimen types, collection materials, transport requirements, and rejection criteria.
 - d. All reagents and equipment – including sources, and concentrations of stocks and working solutions.
 - e. Descriptions of controls and calibrators – including preparation, concentration, and storage.
 - f. Algorithm for interpretation and reporting of all possible results – including examples of any required calculations.
 - g. Technical limitations, potential sources of error, and trouble-shooting protocols.
 - h. Description of standard molecular workflow, separation of pre- and post-amplification areas, and procedures to minimize contamination.
 - i. Reporting of positive results for *Orthopoxvirus* testing.

All positive results must be reported immediately by phone to the local health department (LHD) in which the patient resides. Contact information is available at:
https://www.health.ny.gov/contact/contact_information.

If you are unable to reach the LHD where the patient resides, please contact the NYSDOH Bureau of Communicable Disease Control at 518-473-4439 during business hours or 866-881-2809 evenings, weekends, and holidays.

Additional guidance on controls:

Positive Control – monitors performance of the entire assay and therefore should be run through the entire assay. Plasmid or a synthetic control may be used as a positive control for these assays.

Commercial sources of positive monkeypox control material for molecular assays include: ATCC (<https://www.atcc.org/>) and BEI (<https://www.beiresources.org/>). Additional sources of control material are being produced by several manufacturers and should be available shortly. This document will be updated when the control material is available.

Negative Control – monitors for contamination that could occur at any point during the assay and therefore should be included through the entire assay. Should consist of known negative specimen of same matrix as that being tested. The use of carriers (tRNA, glycogen, DNA, etc) can be used to assist with the detection of low-level contamination.

Inhibition Control – detects the presence of PCR inhibitors in the specimen. May be exogenous spiked nucleic acid, detected in a separate assay, endogenous housekeeping gene. Alternatively, data demonstrating absence or extremely rare (< 0.2%) rate of inhibition with the specimen type being tested and the extraction method being used.

Please also provide:

3. A sample requisition form.
4. Example test reports.
5. A list of any relevant literature (e.g., if using a previously published method).
6. Validation data including the following:
 - a. Summary – including a brief description of the samples used and the experiments performed for assays validation.
 - b. Specificity testing – A list of all organisms tested against – including genetically related organisms or agents that may be expected to be present in the sample type(s) for which approval is being requested. Wet testing should be performed at 10E6 gc per ml or higher. Note: *in silico* alignments are acceptable for specificity testing of other pox viruses.
 - c. Sensitivity testing – at a minimum, LOD experiments should be performed on triplicate replicates of 10-fold serial dilutions, each tested in duplicate, on pooled sample matrix. The LOD should be confirmed by testing 20 replicates at the LOD concentration in 20 individual specimens, then 5 each in 2X, 3X, and 5X the LOD. Note: sensitivity must be established separately for each specimen type.
 - d. Demonstrate inter- and intra-assay reproducibility with

- i. At least 3 clinical or spiked clinical samples run on 3 different days, on different platforms/instruments, by different users.
- e. Accuracy verification – Provide data for at least 30 positive and 30 negative samples, showing raw data. Contrived specimens can be used for accuracy studies. Briefly explain any discrepant results.

Please note:

Following approval of the submitted assay, all positives and first 5 negative samples tested with the assay must be submitted to the Wadsworth Center for confirmation within 7 days.

SECTION 3: CHECKLIST FOR MODIFICATION OF AN FDA OR NYS-APPROVED ASSAY

The submitted package must be organized as numbered or tabbed attachments with all pages individually numbered.

Please provide the following:

1. A brief summary of the changes made to the assay.
2. A standard operating procedure as described in Section 2.2 above.
3. Sample test requisitions and reports as described in Sections 2.3 and 2.4 above.
4. Reference pertaining to the modification if appropriate.
5. Provide the following comparative experiment between the original assay and the modified assay to demonstrate that the modification has not adversely impacted the sensitivity (note – must be repeated in all specimen types and all extraction methods for which approval is being requested):
 - a. Prepare a dilution series of positive control material (at least 6 point) that goes just beyond extinction.
 - b. Perform 5 extractions of each dilution.
 - c. Test each extraction in duplicate.
 - d. Additionally, test 15 negative and 15 positive specimens in modified assay and comparison assay, submit raw data, as well as collated comparison data in a 2x2 table. Explain any discrepant results.

Please note:

Following approval of the submitted assay, all positive and the first 5 negative samples tested with the assay must be submitted to the Wadsworth Center for confirmation within 7 days.