NEW YORK STATE | Department of Health | Wadsworth Center

APPROVAL OF MICROBIOLOGY NUCLEIC ACID AMPLIFICATION ASSAYS

Please submit all information as outlined below. Submit one hard copy of the entire package and one electronic copy (as a PDF file on a CD or flash drive) to:
US Postal Service: Clinical Laboratory Evaluation Program, Biggs Laboratory, Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, NY 12237; Attn: Assay Validation Review
UPS, FedEx, Courier: Clinical Laboratory Evaluation Program, Biggs Laboratory, Wadsworth Center, New York State Department of Health, Dock J - P1 Level, Empire State Plaza, Albany, NY 12237; Attn: Assay Validation Review
Materials submitted, including related data packages, will not be returned to the laboratory. 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"; "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 (Test) Name (e.g., detection of *E. coli*): _____

Target Population (if applicable):_____

Methodology (e.g., PCR; Sequencing, RFLP): _____

Analyte(s) included: _____

Validated Specimen Type(s) (e.g., blood, urine) _____

Clinical Purpose (e.g., detection, quantification): _____

Laboratory Director/Assistant Director (NYS Certificate of Qualification Holder for applicable Permit category)

CQ Code_____Signature _____

Laboratory Director (if not the responsible CQ Holder for applicable Permit category)

CQ Code_____Signature _____

Check One	Assay Type	Necessary Forms for Package Submission
	Laboratory developed test that does not utilize Analyte Specific Reagents (ASRs)	Full validation package-See Section 2
	Laboratory developed test that utilizes Analyte Specific Reagents	Full validation package-See Section 2
	Kit labeled for Investigational Use Only (IUO) or Research Use Only (RUO)	Full validation package-See Section 2
	FDA-cleared/approved assay	DOH 3519f "Notification to Add/Delete FDA-Approved Tests" Form
	Modification of a FDA or NYS-approved assay	Major modification- Full validation package-See Section 2 Minor modification- See Section 3
	Addition of an assay under an approved exemption	Provide the Project ID from your original exemption approval letter, a description of the original exemption, the name of the assay to be added, a summary of the validation performed, and sample reports for all possible outcomes. Attach additional materials as necessary.

SECTION 2: INSTRUCTIONS FOR SUBMITTING A FULL VALIDATION PACKAGE

The checklist below is a guide for items that must be included in the full validation submission packages. This checklist is applicable to nucleic acid amplification tests for infectious agents. The procedure and validation requirements for some specialized tests such as sequencing, subtyping, or prognostic viral typing assays may differ. See Sections IV through VI for additional information. The information submitted must be organized as numbered or tabbed attachments. If an item is not included, indicate the reason. Indicate the **page numbers, appendices and/or tabs where** the items and/or attachments can be found. SUBMISSIONS THAT ARE NOT ORGANIZED AS DESCRIBED MAY BE RETURNED AND THE REVIEW SIGNIFICANTLY DELAYED.

Section 2.1: Standard Operating Procedure And Controls

Page/Tab	
_____	Overview including: • scientific basis of the test and an explanation of the assay • target gene or region being amplified with the location of primers and probes • oligonucleotide list with sequences • target population of the assay and clinical validity
_____	Procedure in compliance with **Operating Procedures Sustaining Standard of Practice 2 (SOPM S2): Content**, including: • detailed step-by-step protocol • specimen collection (where applicable), processing and storage requirements • acceptable specimen types and collection materials (i.e. tube types), specimen transport requirements (i.e. temperature, time to receipt) and specimen rejection criteria • sources of reagents and equipment including: concentrations and volumes of stock and working reagents • a description of all controls and calibrators used in the assay including the function of each control (see additional description and guidance below). Preparation, concentration and storage requirements of controls should be included. Note the frequency of use in the testing protocol as well as acceptable control limits and action(s) to be taken when controls exceed the defined tolerance limit. • algorithm for defining all possible results and procedures for interpreting or reporting each type of result (ex positive, negative, quantitative values, indeterminate, invalid and inconclusive) • examples of all calculations needed to produce interpretable results. • technical limitations of the assay, potential sources of error, and trouble-shooting protocols, and any other information relevant to performing the assay • a description of standard molecular workflow and the strategy used for separating pre-and post-amplification areas, as well as the implementation of methods to minimize amplicon contamination (such as the use of unidirectional workflow and aerosol- resistant pipette tips)

Guidance: The following controls are necessary for infectious agent nucleic acid amplification tests. The procedure should contain details on how each of the following functions is controlled in the assay, including the composition of the control and the step in which the control is included in the assay. Additional guidance on acceptable control materials and procedures is provided below:

- **Positive Lysis/Extraction Control**
  The positive lysis/extraction control will monitor the performance of the entire assay, including the lysis/extraction process, to ensure that it is performing as expected. This control should contain whole organism (bacterial cells, viruses, parasites, fungi) or, if these are unavailable, nucleic acid. This positive control should be included at a low but easily detectable concentration, and should be run through the entire assay.
- **Negative Lysis/Extraction Control**
  A negative lysis/extraction control will monitor potential contamination that could occur during the entire assay, including the lysis/extraction process. This control should be run through the entire assay (lysis, extraction, and amplification). Ideally, this control should consist of a known negative specimen in the same matrix that is being tested. The use of carriers such as tRNA, glycogen, or DNA can be used to detect low level nucleic acid contamination.
- **Inhibition Control**
  An inhibition control ensures that patient samples are free of amplification inhibitors, which could lead to false negative results. In some cases, the inhibition control may also serve as an extraction control. In all cases, an expected value or range should be developed for the inhibition control, and results should be interpreted accordingly. If inhibition is tested using exogenous spiked nucleic acid, controls must be included to monitor for contamination with this material.
  Inhibition controls are not required if the run includes isolates only and not primary patient specimens.
  Examples of inhibition controls include, but are not limited to:
  -specimens spiked with an exogenous nucleic acid control that is detected in a separate assay
  -specimens spiked with a control nucleic acid containing primer-binding sites identical to the target, but with a heterologous probe-binding internal sequence
  -specimens dispensed into at least two aliquots tested in parallel; one aliquot is spiked with a low level of a positive or inhibition control
- Alternatively, data must be provided that demonstrate the absence (or only rare occurrence) of inhibition, in each specimen type. If the extraction method is well established, with supportive evidence in the peer-reviewed literature, these references can be submitted together with the laboratory's own validation data. For each specimen type, the data set should include a total of 500 hundred drawn from the literature, plus 100 samples directly tested in the submitting laboratory that demonstrate that the method is performing according to expectations. The specimens tested in the submitting laboratory should contain low but easily detectable amounts of target. Specimens should be extracted and assayed, with results provided to show no loss in detection.
- If independent supportive evidence is not available, the laboratory needs to provide more extensive in-house data. Typically, 500 samples are adequate to verify that inhibition is occurring in less than 1% of the samples. It should be noted that it is acceptable to obtain data assayed from the same matrix containing different but similar analytes if it is needed to attain the required number of samples.
- **Reagent Contamination Control**
  A reagent contamination control (i.e. no-template control) ensures that the amplification reagents are not contaminated. This control should consist of water or buffer in a reaction tube that contains all of the reagents for amplification, but does not contain nucleic acid template.
- The inclusion of additional negative controls is strongly recommended whenever the prevalence of the target agent is routinely expected to be high, e.g., in HCV screening of intravenous drug users, or patients with suspected hepatitis.

Section 2.2: Test Requisition And Reports

Page/Tab	
_____	A sample requisition form containing all the required elements in **Requisition Sustaining Standard of Practice 4 (Requisition S4): Request Form**.
_____	Examples of test reports containing all findings (e.g. positive (quantitative or qualitative), negative, indeterminate, inconclusive, etc.) with interpretive text, assay limitations and any disclaimers required by the federal government for tests utilizing Analyte-Specific Reagents (ASRs) and in compliance with **Reporting Sustaining Standard of Practice 1 (Reporting S1): Report Content)**. If preliminary or presumptive positive results will be reported without confirmation, include examples of these reports containing the appropriate statements explaining the presumptive/preliminary nature of the results.

Section 2.3: References

Page/Tab	
_____	A list of relevant literature references that describe the scientific basis and clinical validity of the assay. Provide copies of only primary references.
_____	Applicable package inserts

Section 2.4: Validation

Page/Tab	
_____	**Validation Data Summary**: Succinctly summarize why this new assay is needed, the means by which the new assay was validated, and the results. This should include a description of testing (authentic patient or spiked samples and total number), comparison method (gold standard or other FDA or NYS-approved assay) and overall results. Authentic clinical specimens are preferred, but spiked clinical samples are acceptable.
_____	**Specificity:** • Provide a list of all organisms tested in the specificity study and the verification run, including the source and concentration of each organism or nucleic acid target if whole organism is not available. • Provide the results of the specificity study. If there is any cross-reactivity, provide additional information on how the results will be resolved or interpreted.
	Guidance: • Specificity of the assay should be demonstrated using genetically-related organisms, organisms that can produce similar symptoms or illness, and other organisms that can be present in the specimen matrix/matrices to which the assay will be applied. If any cross-reacting organisms are noted, they should be clearly specified in the application, as well as on the patient report of test findings. In addition, assay a minimum of 5 different strains/isolates of the intended target organism/virus if available. If there are additional genotypes/serotypes/subtypes/variants, these should be included as well. • Molecular amplification assays for microbial detection that are not probe-based, such as SYBR® Green or real-time PCR with melting curve analysis, typically require the use of a secondary method in order to report a result as confirmatory. These assays can be used as screening assays but must be confirmed with an alternative method, i.e., a probe-based, hybridization-based, or sequence-based method. In the absence of such confirmation, positive results are considered presumptive, and this should be clearly indicated on the report (Also see Accuracy Verification section below).
_____	**Sensitivity:** • Provide a brief description of experiments used to demonstrate the limit of detection (LOD) of the assay. • Provide the results of the LOD experiments for each specimen matrix.
	Guidance: **There are various methods for the determination of the LOD. Methods that statistically determine the LOD with 95% confidence are acceptable. Listed below are the minimum accepted requirements. All LODs determined from primary matrices must be performed from extraction.**

	• **Primary Matrix** Provide data from a dilution series of a known concentration of the target (preferably whole organism) that is spiked into negative clinical samples for each matrix being validated. Perform 3 separate extractions of this dilution series and test in duplicate. The dilution series must encompass six dilutions including one below the limit of detection of the assay. If the LOD is determined to be > 100 gene copies, linearity data must be provided. • **Multiple Specimen Matrices** When an assay is validated in multiple sample types (matrices), a separate dilution series should be performed for each matrix, although some sample types may be grouped. If it is unclear whether you need to independently spike different matrices (for example, different types of body fluids) please contact CLEP for advice. • **Microbial or Viral Isolates** Provide data as stated above for primary matrix except isolates may be diluted in water or buffer. • **LOD Requirements for Virology Applications** For virology applications, the LOD in genomic copy number is required. For quantitative viral assays, the limit of quantitation (LOQ) should be determined. Quantitation based on plaque titrations of viruses is not acceptable. It is acceptable to perform 1:10, 1:100, or 1:1000 dilutions of specimens that exceed the validated upper limit of quantification provided that the diluted specimen falls within the acceptable reportable range and internal controls are used to ensure that the diluent does not introduce inhibitors.
_____	**Inter-assay Reproducibility:** • Provide a brief experimental description and results demonstrating inter-assay reproducibility.
	Guidance: At least 3 authentic clinical samples or spiked clinical samples should be run on three different days. If spiked, the three samples should include one concentration at or near the limit of detection. Alternatively, a single positive control run repeatedly over many days (15 or more) may be used.
_____	**Intra-assay Reproducibility:** • Provide a brief experimental description and data demonstrating intra-assay reproducibility.
	Guidance: At least 3 authentic clinical samples or spiked clinical samples should be run in triplicate. If spiked, the three samples should include at least one concentration at or near the limit of detection. Also, if different instruments, platforms, models or technicians will be used to perform the assay, demonstrate the assay's consistency across these variables.
_____	**Accuracy Verification:** • Provide a description of the validation study design. See guidance below for specific details. • Provide data from *at least* 30 positive samples and 10 negative samples for each specimen type together with controls used in the assay. Preferably present the results in tables (2 x 2), showing the qualitative results compared to results obtained by the comparison method. • For each assay, provide the number and type of specimens tested, including information on the subtypes, genotypes, etc tested in the assay. If multiple clinically relevant sub-types or genotypes are available for the organism being assayed, please include an example of each available type, or a representative range of subtypes. • Provide a brief summary of the results, including an explanation of any discrepant results and how the discrepancy was resolved. • Submit one representative example of test results (one high-quality original printout of an actual test run), a condensed summary of the raw data (such as Ct values), and a complete description of how all results were interpreted. • Molecular amplification assays for microbial detection that are not probe-based, such as SYBR Green or real-time PCR with melting curve analysis, data must be submitted from a secondary confirmatory method. These assays can be used as screening assays but must be confirmed with an alternative method, i.e., a probe-based, hybridization-based, or sequence-based method. In the absence of such confirmation, positive results are considered presumptive, and this should be clearly indicated on the report. (Also see Specificity validation guidance above).

	Guidance: Specimen characteristics for accuracy verification: • Accuracy should be verified by conducting a randomized, blinded validation study where the assay results are compared to those of a gold standard or FDA or CLEP approved assay, or results of spiked clinical samples that are compared to predicted results based on the spiking values. • If multiple sub-types or genotypes are available for the organism being assayed, please include an example of each type, or a representative range of subtypes. As many different specimens and different strains, types, or clinical isolates as possible should be used in spiking studies (especially for specimen matrices that are routinely tested and pathogens that are routinely isolated in the laboratory). If your laboratory has difficulty in obtaining sufficient samples of a particular specimen type, or any other problems in fulfilling the validation requirements, please contact CLEP for guidance. • The samples should be authentic clinical specimens, but spiked samples are acceptable when clinical specimens are not available. If spiked samples are used, at least 10 samples should be close to the LOD (maximum, 10-fold above that level). If authentic clinical samples are used, at least 10 of the 30 positives should be weak positives, if the methods used allow for this determination. Include multiple different strains and specimens. • For quantitative assays, please provide data across the full range of concentrations likely to be encountered in clinical samples. • Any discrepant results need to be explained. • Multiplex assays (especially those with multiple sample types) can present a difficult situation for the design of a validation study, since it can be a large and expensive endeavor to assay 40 samples (30 positive and 10 negative) for each analyte in each specimen type. Samples can be spiked with multiple organisms, so as to reduce the number of samples required. CLEP may be contacted for guidance prior to the submission of a package with multiplex analysis having four or more simultaneous targets.

Section 2.5: Quality Assurance

Page/Tab

_____	A plan for proficiency testing including criteria for passing, the number of samples included in the proficiency panel, and the corrective actions that would be taken in the event of inadequate verification of the assay and individual analyst performance, compliant with **Quality Assessment Sustaining Standard of Practice 3 (QA S3): Ongoing Verification of Examination Accuracy**

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

The checklist below is a guide for items that must be included in the method validation submission packages for minor modifications of an FDA or NYS- approved assay such as a change in matrix or specimen volume. Any major modification to an assay such as in a change in primer, probe or target gene would require a full validation. A change in intended use, such as using a quantitative assay (prognostic) for qualitative detection (diagnostic), requires a full validation. For additional information on full validations, please refer to Section II for guidance on how packages should be organized and what specific information should be included in each submission. The information submitted must be organized as numbered or tabbed attachments as indicated below. If an item is not included, indicate the reason. **SUBMISSIONS THAT ARE NOT ORGANIZED AS DESCRIBED MAY BE RETURNED AND THE REVIEW SIGNIFICANTLY DELAYED.**

Section 3.1: Summary Of Modifications Made To The Approved Assay

Page/Tab	
_____	Provide a succinct summary of the changes made to the approved assay.

Section 3.2: Updated Assay Information

Page/Tab	
_____	Standard operating procedure (as defined in Section 2.1 above)
_____	Sample test requisitions and reports, if changed (as defined in Section 2.2 above)
_____	References pertaining to the modification, if appropriate.

Section 3.3: Validation

Page/Tab	
_____	**Sensitivity:** Provide data demonstrating the limit of detection (LOD) of the assay <u>in each specimen matrix</u> to which the assay will be applied. A side-by-side comparison of the modified assay and the non-modified assay can be performed using a dilution series of organisms <u>in matrix</u> to demonstrate that there is no change in the LOD from the non-modified assay.
_____	**Reproducibility**: (only if appropriate)
_____	**Accuracy Verification:** Data should be provided from a blinded study with *at least* 30 samples including 20 positive samples (with 10 near the limit of detection) and 10 negative samples for each analyte. These samples can be spiked specimens if true clinical samples are not available. However, spiked laboratory diluents or buffers are not acceptable.

SECTION 4: ADDITIONAL INFORMATION FOR BROAD RANGE SEQUENCING ASSAYS

Broad-range sequencing is defined as the use of conserved sequences within phylogenetically informative genetic targets (i.e. 16S rDNA, 28S rDNA, internal transcribed spacer regions, gyrase B etc.) to identify bacteria, mycobacteria, or fungi. For additional information on specific viral sequencing, subtyping, or genotyping applications, please contact CLEP at 518-485-5378 or CLEPVAL@health.state.ny.us.

Section 4.1: Broad Range Sequencing Standard Operating Procedure (SOP) Requirements For Bacteria, Mycobacteria, And Fungi

Page/Tab	
_____	Laboratories that apply for approval to carry out identification and subtyping using DNA sequencing and comparisons to sequence databases will be required to provide the following information in the SOP (using Section II, Section II.1 Standard Operating Procedure and Controls above as a guide).
_____	A complete description of controls that are used in the assay. • A negative amplification control should be included in each assay. • Positive amplification controls are not necessary as all reactions should be positive. In addition, sequencing controls to assess the quality of the sequencing reaction should include both a negative and positive in every run. • The sequencing negative control may be water or buffer and the sequencing positive control could be a purified plasmid that is supplied with some commercially available sequencing kits.
_____	A description of the criteria used to determine the minimum sequence length that is required to properly carry out identification. • For short read lengths, at least 300 bp or full-length target sequence if shorter than 300 bp must be utilized for comparison and must span a region of variability. • CLSI guideline MM18-A can be consulted for further information on sequence data parameters that should be considered in all sequence-based identification assays.
_____	A description of the process used to analyze sequence data, including: • review of electropherograms • the expected read length of the sequence • the protocol used for interpreting ambiguous base calls
_____	A detailed algorithm describing the range of identity matches that will be reported (i.e., genus, species, strain, serotype, or genomovar level including the percent identity).
_____	If database information is proprietary, and other information is not available, an explicit comment should be added in the client education materials, indicating that the database(s) have not been reviewed or approved by the New York State Department of Health.
_____	Sample reports must be submitted detailing all potential outcomes.
_____	The use of a single public database is not acceptable for bacterial sequence-based identification assays. If searches are performed using the BLAST algorithm, identification parameters should be set so that only query results consisting of sequence coverage of 100% along the entire length of the sequence are used for identification. The reference database (commercial or in-house developed) that is utilized must be verified by comparison to results obtained by conventional microbiological methods, and/or by a NYS-approved assay or multiple public databases.

Section 4.2: Validation Requirements of Isolates for Broad Range Sequencing Of Bacteria, Mycobacteria, and Fungi

Section 2.4 Validation and Section 2.5 Quality Assurance should be consulted as guides for the steps used to monitor performance of the assay which should be outlined in the validation material. Below are additional guidelines that need to be followed for proper validation of these assays:

Page/Tab	
_____	If an application includes a sequenced-based assay tested directly from a patient specimen rather than a culture/isolate, sensitivity and specificity data should be submitted.
	Data demonstrating specificity and the limit of detection (LOD) for use on bacterial, mycobacterial, or fungal isolates are not required.
_____	Reproducibility studies should include testing of 3 different authentic isolates tested in singlicate on 3 different days. If possible, each of the 3 authentic isolates should be tested by a different laboratorian.
_____	Data should be submitted from at least 30 representative organisms tested in a blinded fashion, and should include the phenotypic identification of each organism. If 30 representative organisms cannot be obtained, call CLEP (518-485-5378) for guidance.

Data can be submitted in the following format:

Sample #	Commercial Database Match (Organism)	Input/ Seq. Length (bp)	Output/ match Length (bp)	#Mismatch (bp)	% Match/ ID	Sequence Identification	Culture/ Gold Standard Identification	Final Identification
1496	*Ralstonia picketti*	436	436	0	100%	*Ralstonia pickettii*	*Ralstonia pickettii*	*Ralstonia pickettii*
	Ralstonia solanacearum	436	436	19	95.6%			
1644	*Haemophilus parahaemolyticus*	460	460	7	98.5%	*Haemophilus parahaemolyticus*	*Haemophilus parahaemolyticus*	*Haemophilus parahaemolyticus*
	Haemophilus paraphrohaemo-lyticus	460	460	10	97.8%			
1555	*Streptococcus bovis*	468	468	0	100%	These four species cannot be differentiated by 16S rDNA sequence analysis	*Streptococcus bovis* group	*Streptococcus bo*vis group
	Streptococcus lutetiensis	468	468	0	100%			
	Streptococcus infantarius	468	468	0	100%			
	Streptococcus equinus	468	468	1	99.8%			
	Streptococcus alactolyticus	468	468	27	94.2%			

- After satisfactory review of the SOP and validation data, the application will be tentatively approved. The laboratory will then be required to participate in a specialized assessment administered by the Wadsworth Center, consisting of a blinded panel of up to 10 data sets or organisms. Full approval will be granted after successful completion of the assessment. If performance on the specialized assessment is unsatisfactory, CLEP will contact the laboratory to provide guidance, and to discuss additional training necessary in order for the laboratory to obtain approval to perform broad-range sequencing.

SECTION 5: SUBTYPING

Page/Tab	
_____	Submissions for approval to perform subtyping using nucleic acid sequencing and comparisons to sequence databases (which need to be identified and defined): • Provide data on as many different sub-types as possible in the validation panel. • If available, include at least three isolates for each sub-type represented in the database. • If database information is not available, an explicit comment should be added in the client education materials, indicating that the database(s) has/have not been reviewed or approved by the New York State Department of Health. • For additional information on validation requirements for specific viral sequencing, subtyping, or genotyping assays, please contact CLEP (518-485-5378 or CLEPVAL@health.state.ny.us).

SECTION 6: PROGNOSTIC VIRAL TYPING ASSAYS

There are a wide variety of methods and intended uses for viral typing assays such as genotyping and serotyping. Viral typing assays are not intended to be used to diagnose infection but rather to characterize the virus after it has been detected using a test that has been approved for detection or diagnosis. The following modifications to the items in Section 2 should be considered for viral typing assay submissions.

Section 6.1: Standard Operating Procedure and Controls

Page/Tab	
_____	Overview including: • A description of how the results will be used (patient management, epidemiologic information etc.). • Indicate whether the amplification product that is analyzed in the typing assay originated from another assay such as a detection assay or a quantitation assay. •
_____	Standard operating procedures and controls (as defined in Section 2.1 above) • Requirements for controls are generally the same as in Section 2.1, that inhibition controls may be excluded

Section 6.2: Test Requisition And Reports

Page/Tab	
_____	Test reports should include an interpretation of the results such as prediction of drug resistance or virulence and there should be supporting documentation to justify the interpretation of test results.

Section 6.3: References

Page/Tab	
_____	Same as in Section 2.3 above.

Section 6.4: Validation

Page/Tab

_____	**Validation Summary-** Same as in Section 2.4 above
_____	**Specificity:** • Viral typing assays that are based on sequencing do not require specificity data. • Viral typing assays that are based on probe hybridization or other non-sequencing methods require specificity data if the amplification reaction has not been validated for specificity.
_____	**Sensitivity:** • The limit of detection of the assay should be determined in the specimen matrix by preparing a dilution series of three specimens each containing a different virus type (e.g. genotype, subtype, variant) of a known viral genome copy number. The full assay should be conducted, but it is not necessary to perform the assay in duplicate for each set of dilutions. •
_____	**Reproducibility**: • Viral typing assays require reproducibility data as described in Section 2.4 above
_____	**Accuracy Verification:** • The blinded panel should consist of at least 30 positive samples for each specimen type. It is not necessary to include any negative samples if the assay is only performed on known positive samples. For assays that determine a virus genotype, it is preferable that the panel include several representatives of each different genotype that will be reported. For assays that identify drug resistance mutations, the panel should contain samples with a range of different results including samples with different resistance mutations and some with no mutations. • It is understood that certain genotypes and mutations are very rare may be difficult to obtain; however, validation of the assay to accurately identify these genotypes must be performed if they are to be reported. If rare genotypes are not available, in some instances, reports will need to include a statement indicating the limitations of the assay and specifying the genotypes that may be reported when sequence confirmation is not available. It is also acceptable to use synthetic material to verify rare genotypes and mutations. Ongoing validation of rare genotypes may be conducted and when additional data are available, they may be submitted to CLEP for review. Additionally, the SOP must contain written procedures for handling results indicative of a genotype that has not been fully validated. This may include referral to another laboratory. • For genotypes with multiple subtypes, different subtypes should be included in the validation study if possible; however assay verification of all subtypes may not be feasible and is not required. • If specific subtype results would be used in clinical management of the patient, the relevant subtypes must be included in the verification study.
_____	**Mixed genotypes** • In some cases, a patient may be infected with viruses of two different genotypes. Some typing methods, such as probe-based methods, are capable of detecting mixed infections. If the method may detect more than one genotype in a patient sample, the procedures must include instructions on how those results will be handled. If mixed infections will be reported, proper assay verification needs to be performed. Assay verification studies may be performed by preparing mock specimens containing mixtures of viruses of known genotype at various concentrations. For example, two pure samples can be used to prepare mixes of the genotypes at various ratios (e.g., 1:1, 2:1, 1:2, 4:1, 1:4). If detection of mixed genotypes is not validated, the SOP must contain instructions on how the result will be reported (i.e. inconclusive, mixed infection detected).

SECTION 7: RESUBMISSIONS

Once the package has been reviewed, the laboratory will receive either an approval letter or a letter requesting additional information. In order to facilitate our response to resubmissions, we request that these be as clear and well organized as possible. Please provide a point-by-point response to the reviewers' comments, as well as the appropriate supporting documentation. If revisions to the procedure manuals were requested, please submit entire new versions, and clearly indicate where the changes were made.