NEW YORK STATE | Department of Health
Wadsworth Center

APPROVAL OF SEROLOGIC ASSAYS FOR INFECTIOUS DISEASES AND AUTOIMMUNE DISORDERS

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. Materials are subject to New York State's Freedom of Information Law (commonly called FOIL). We suggest marking your documents as “proprietary” or “confidential”. If so marked, laboratories will be given an opportunity to block information release.

SECTION 1: GENERAL INFORMATION

Laboratory Name: _____ NYS PFI: _____

Assay (Test) Name (e.g., detection of serum antibodies to West Nile virus):

_____

Assay (Test) Identifier(s) (e.g., Test Code(s)):

_____

Assay (Test) Type: ☐ Laboratory Developed Assay (LDA) ☐ Investigational Use Only (IUO)
☐ Analyte Specific Reagent (ASR) ☐ Research Use Only (RUO)

For RUOs only, provide the manufacturer/kit used: _____

Target Population (if applicable): _____

Methodology (e.g., ELISA, MIA, IFA): _____

Analyte(s) included (West Nile Virus IgM, etc.):

_____

Validated Specimen Type(s) (e.g., serum, plasma, CSF):

_____

Clinical Purpose (e.g., aid in the diagnosis of West Nile Virus infection):

_____

All submissions must include a “Risk Attestation Form” found on the CLEP Test Approval webpage.

If this submission is a modification of an FDA or NYS-approved assay, see the “Assay Modification Checklist” on the CLEP Test Approval webpage.

If this submission is for the addition of an assay under an approved exemption, see the “Add Test Under Exemption Checklist” on the CLEP Test Approval Webpage.

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 antibody detection tests for infectious agents and autoimmune disorders. The information submitted must be organized as **numbered or uniquely named attachments**. If an item is not included, indicate the reason.

Section 2.1: Standard Operating Procedure and Controls

File Name	
	Overview including: • scientific basis of the test and an explanation of the assay • specialized requirements/conditions for using the assay • target population of the assay and clinical validity
	Procedure in compliance with **Test Procedure Content Standard of Practice 1 (TPC S1): Test Procedure 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 transportrequirements (e.g., temperature, time to receipt) and specimen rejection criteria. • sources of reagents and equipment including concentrations and volumes of stock and workingreagents including antibodies and antigens used in the assay. • 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. • the algorithm for defining all possible results and procedures for interpreting or reporting each type of result (e.g., 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, trouble-shooting protocols, and any other information relevant to performing the assay
	Guidance: The following controls are necessary for serological assays for infectious and autoimmune disorders. The procedure should contain details on how each of the following functions is controlled in the assay, including the composition of the control and where the control is included in the assay. Controls should be run through the entire assay. • **Positive Control** The positive control should be included at a low but easily detectable concentration. Spiking may be acceptable in exceedingly rare cases but should not be used as a substitute for clinical samples in most instances. For quantitative assays, a low (near the lower limit of detection) and a high (near the upper limit of detection) positive controls are required. • **Negative Control** This control should consist of a known negative specimen in the same matrix that is being tested. The inclusion of additional negative controls is strongly recommended whenever the prevalence of antibodies is routinely expected to be high. • **Blank/buffer/background Control** Blank measurements that serve as reference measurements of the buffer/matrix of the sample.

Section 2.2: Test Requisition and Reports

File Name	
	A sample requisition form containing all the required elements in **Test Request Standard of Practice 3 (TR S3): Test Request Form.**
	Examples of test reports containing all findings (e.g., positive, 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 Standard of Practice 2 (REP S2): Test 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 and recommendations for confirmation, if applicable.

Section 2.3: References

File Name	
	A list of relevant literature references that describe the scientific basis and clinical validity of the assay. Provide copies of only primary references for non-traditional analytes only.
	Applicable package inserts

Section 2.4: Validation

File Name	
	Validation Data Summary: Succinctly summarize why this new assay is needed, how the new assay was validated, and the validation results. This section should include a description of testing, number of samples used for validation, comparison method (gold standard or other FDA or NYS-approved assay) and overall results.
	Analytical Specificity: • Provide a list of all specimens, including specimen types tested in the specificity study. • Provide a summary of 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 by including at least 30 specimens from confirmed similar diseases or conditions. In addition, include at least 10 positive specimens from confirmed autoimmune disorders (e.g., systematic lupus erythematosus, rheumatoid arthritis, multiple sclerosis). If any cross-reacting sera are noted, they should be clearly specified in the application. These specimens may count towards the criteria for Assay Verification studies using at least 50 negative specimens (see Assay Verification section below). • The summary must explain discordant results.

	Analytical Sensitivity
	• For qualitative assays:
	  • Assays detecting antibodies, a side-by-side dilution series compared to an FDA or NYS approved assay is required if such assays are available.
	  • Provide a summary of the sensitivity study results for each type of specimen matrix.
	• For quantitative assays:
	  • Provide a summary of the sensitivity study results for each specimen type.
	  • Analytical sensitivity measures the smallest quantity of an analyte that can be reproducibly distinguished from background levels or a zero calibrator in each assay system. It is also called the limit of detection (LOD) of the assay. LOD is defined at the 0.95 confidence level (+/- 2 standard deviations).

	Guidance: • For qualitative antibody detection assays, serially dilute at least 3 specimens and run them in the established assays and with the new assay. The results should be concordant with the established assay. • For immunological detection/quantitation of antibodies, test at least 3 different specimens with a known concentration of the antibody and serially dilute each through the analytical range and beyond the expected detection limit.

	Reference interval (normal range):
	• Depending on the assay type, the reference interval (RI) may be:
	  • A set of numerical ranges that are assigned to a qualitative result for a semi-or quasi-quantitative assay
	  • Numerical range for a quantitative assay

	Guidance: • Reference intervals should be determined using specimens from a clinically relevant population. • Reference intervals are expected to be characteristic of 95% of healthy subjects (50% male and 50% female, if applicable) tested for the analyte. To establish a reference interval, it is recommended to collect data from 120 samples (60 male and 60 female) that are representative of the population encountered by the clinical laboratory. Use a simple nonparametric method to analyze the data. If fewer than 120 samples are used, traditional parametric methods should be used for estimation i.e., Horn and Pesce method.
	Reportable range (if applicable): • For quantitative detection, provide the range of values that lie between an established upper and lower limit for the analyte of interest and include the upper and lower limits.
	Guidance: • Use 8 to 12 concentrations across the anticipated measuring range. Replicate the test at least 3 times. This can be done by doing a linearity study and/or combined with the sensitivity study.

	Other characteristics required for test performance (if applicable):
	• Provide a brief experimental description and results demonstrating other characteristics required for test validation such as:
	  ○ Cutoff establishment
	  ○ Sample stability
	  ○ Carryover studies
	  ○ Immunoglobulin class specificity

	Guidance:
	• **Cutoff establishment:**
	  ○ Generally cutoff is equal to the mean of the negative samples plus 3 standard deviations.
	  ○ Can also be calculated based on ROC (receiver operating characteristic) analysis.
	  ○ If appropriate, the cutoff value should be determined for relevant geographical regions or population and for each disease condition.
	• **Sample stability:**
	  ○ Evaluate stability of relevant specimen matrices under various storage and shipping conditions such as room temperature, elevated ambient temperatures, freezing/thawing (e.g., 2-8 ℃) for 15 and 30 days or the time frame specified in the SOP.
	• **Carryover:**
	  ○ Select one high positive sample that must be tested in a series alternating with one negative sample in a carryover study.
	• **Class specificity:**
	  ○ The possibility of cross-reactivity between classes of antibodies should be evaluated. For IgM assays, high level of IgG for the analyte in question should be tested to determine specificity.
	  ○ Show verification of class specificity of the detection reagent. If commercial sources of antibodies are used for detection, please provide the manufacturer's information on class specificities.
	• **Multiple Specimen Matrices**
	  ○ When an assay is validated in multiple specimen types (matrices), the performance characteristics of the test may need to be established for each specimen type.

	Reproducibility: Provide a brief experimental description and summary of results demonstrating both inter-assay and intra-assay reproducibility. The summary must include a calculation of the standard deviation, coefficient of variation and confidence interval.
	Guidance: • **Inter**-assay reproducibility: At least 2 separate runs should be performed that include least five (5) specimens each of strong, intermediate, and weakly reactive specimens. A negative specimen must also be tested. • **Intra-**assay reproducibility: Strong, intermediate, and weak positive specimens should be used. At least 1 positive clinical sample from each category and a negative should be tested on the same run. • If different instruments or platforms will be used to perform the assay, demonstrate the assay's consistency across these variables.
	Accuracy Verification: • Provide a list of all specimens and specimen types tested in the verification run. • Accuracy should be verified by conducting a randomized, blinded validation study where the assay results are compared to those of an FDA or NYS approved assay. The samples should be authentic clinical specimens, but spiked samples are acceptable when clinical specimens are not available. Provide a brief summary of the results (a 2x2 table is preferred), including an explanation of any discordant results and how this was resolved. • Provide a condensed summary of the raw data and a complete description of how all results were interpreted. • If applicable, submit one representative example of test results (e.g., one high-quality original printout of an actual test run).

	Guidance:
	Specimen characteristics for accuracy verification:
	• Provide data from 50 each of strong, intermediate, and weakly reactive specimens and 50 negative samples for each specimen type together with controls used in the assay. For rare diseases, lesser numbers of samples may be acceptable. Preferably present the results in Summary 2x2 contingency tables, showing the qualitative results compared to results obtained by the comparison method.
	• Perform statistical analysis for quantitative assays:
	  • xy scatter plot with regression analysis
	  • Bland-Altman difference plot with determination of bias
	  • % agreement with kappa statistics
	• Please provide data across the full range of concentrations likely to beencountered in clinical samples.
	• 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. Contact clepval@health.ny.gov with a detailed description of your assay and specific questions if needed.

Section 2.5: Quality Assurance

File Name

	Proficiency Testing: 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. The proficiency testing plan must be compliant with **Proficiency Testing Standards of Practice.** If there are no commercially available proficiency testing programs for the analyte(s), a detailed plan for an alternative to proficiency testing must be provided. This plan must be in compliance with **Proficiency Testing Standard of Practice 3 (PT S3): Alternative to Proficiency Testing.**

	Systems agreement: If different instruments or platforms will be used to perform the assay, demonstrate the assay's consistency across these variables.
	Quality Control Plan: Identify the critical steps in the test procedure and the quality control measures taken to control and monitor assay performance for consistent and reliable results.

Section 2.6: Test Design

The below list of questions **must** be answered for **ALL** validation submissions. NOTE: Any automated software, algorithm, statistical models, or simpler artificial intelligence (AI) methods MUST be locked. Unlocked algorithms will not be considered.

Does this assay use an automated software, algorithm, statistical models, or simpler AI methods (e.g., decision trees, logistic regression)? ☐ Yes ☐ No

If the above answer is yes, please complete the below section. Below, the term 'algorithm' refers to any automated software, algorithm, statistical models, or simpler AI methods:

Is the algorithm locked? ☐ Yes ☐ No

Was the algorithm developed in-house? ☐ Yes ☐ No

If developed in-house, please complete the section below:

File Name

	NARRATIVE SUMMARY of the data used to train and validate the algorithm. If you refer to previously published information on the cohort, please provide the full reference. The **NARRATIVE SUMMARY** must include the following: • Sample size • Inclusion/exclusion criteria • Data quality control policy(ies) • Relevant patient characteristics (e.g., age, gender, ethnicity), as applicable • The software package(s) used • How the algorithm's features or variables were selected • Description of any algorithms, statistical models, or simpler AI methods implemented by the software • Indicate if advanced AI/ML, such as Natural Language Processing (NLP) was used in the development process • Where/how the training/validation data was generated (e.g., in-house, previously published)
	Provide the power or sample size calculations used to determine specimens or data points required for training/validation. Include justification for the calculations.
	What quality control processes do you have in place? Describe any pre-analytical (e.g., specimen rejection criteria) and analytical controls to monitor the software/algorithm/model output.
	Explain how the algorithm is locked from revisions during use.
	What information do you provide to clinicians that allows them to make an independent decision about the results? Include details on the data set, key variables or analytes, and error metrics such as accuracy, precision, recall, and any misclassification rates.