Expert Recommendations for CLDN18.2 Biomarker Implementation

With the emergence of new biomarkers in gastric/gastroesophageal (G/GEJ) cancer, it is critical that pathology laboratories understand and adopt best practices related to immunohistochemistry (IHC) assay testing.

Developed by a committee of pathology experts, this guide is designed to help laboratories onboard testing for CLDN18.2.

1 Recommendation Highlights: Principles of Analytic Validation of IHC Assays[1,2]

NOTE: The following recommendations are applicable to IHC testing in G/GEJ cancer.

RECOMMENDATION 1

Laboratories must validate all IHC tests before placing into clinical service.

Validation sets should include positive, negative, and borderline expression results:

- **Positive control –** Tissues that contain the antigen of interest expressed at levels above a threshold
- **Negative control –** Tissues that do not contain the antigen of interest

Normal tissues should not comprise the entire validation set.

Validation sets should reflect the intended use of the assay, as well as the indication of interest (i.e., gastric).

RECOMMENDATION 2

For initial validation of clinically-used assays, at least 90% concordance should be achieved between the new assay and the comparator assay.

If concordance is less than 90%, the cause should be investigated.

RECOMMENDATION 3

For initial analytic validation of predictive biomarkers, 40 validation cases (20 positive, 20 negative) should be used.

If the pathologist determines that fewer than 40 validation cases are sufficient, the rationale for that decision needs to be documented.

Laboratories may choose to use tissue sets with larger than the recommended minimum number of cases.

If validation results do not meet the 90% concordance standard, the basis for this result and appropriate mitigation (testing of additional tissues, change in test conditions) should be determined.

RECOMMENDATION 4

When possible, laboratories should use validation tissues processed using the same fixative and processing methods as cases to be tested clinically.

Tissues included in the validation set must be representative of the specimens to be received in routine practice.

Validation set must provide a representative range of expression intensities and patterns.

RECOMMENDATION 5

Laboratories may use whole sections, tissue microarrays (TMAs) and/or multi-tissue blocks (MTBs) in their validation sets, as appropriate.

Whole sections should be used if TMAs/MTBs are not appropriate for the targeted antigen or if the laboratory medical director cannot confirm that the fixation and processing of TMAs/MTBs is similar to clinical specimens.

TMAs/MTBs contain multiple previously tested positive and negative tissues (allows for comparison of results in multiple tissues tested with an identical assay protocol).

TMAs/MTBs have limitations:

- Biomarkers with high-levels of heterogeneity (PD-L1, gastric HER2)
- Due to the small size of each tissue sample, TMAs/MTBs usually have less antigen-positive cells and negative control cells than whole sections
- Expression in TMAs (including normal gastric mucosa as the normal positive control) may not reliably represent the entire tumour
- Limited tissue expression (e.g. BCL-6)

RECOMMENDATION 6

When a new antibody lot is placed into clinical service for an existing validated assay, laboratories should confirm the assay's performance with at least one known positive case and one known negative case.

Confirmation that assay performance has not changed is necessary when a new antibody lot is used.

Laboratories should confirm assay performance through, but not limited to, the following ways:

- **Antibody vendor** (same clone)
- **Antibody dilution**
- **Incubation or retrieval times** (same method)

Confirmation that assay performance has not changed is necessary when there are minor changes to the assay method.

Laboratories may want to include borderline positive expressors:

- 2 positive cases (1 weak and 1 strong) should be considered.

RECOMMENDATION 7

Laboratories should confirm assay performance by testing a sufficient number of cases to ensure that assays consistently achieve expected results.

Laboratories should confirm assay performance when any of the following have changed:

- **Fixative type**
- **Antigen retrieval method** (e.g., change in pH, different buffer, different heat platform)
- **Antigen detection system**
- **Tissue processing or testing equipment**
- **Environmental conditions of testing** (e.g. laboratory relocation)
- **Laboratory water supply**

RECOMMENDATION 8

Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay.

RECOMMENDATION 9

The laboratory must document all validations and verifications in compliance with regulatory and accreditation requirements.

2 Pre-Analytics & Best Practices[3]

METHOD OF GROSS PROCESSING
Section thickness for surgical specimens: ≤5 mm
Mass/volume ratio: ≥4:1 (Optimal: ≥10:1)
Transport temperature: ambient
METHOD OF STABILIZATION
Type of fixative: 10% neutral phosphate-buffered formalin
Time in fixative: 6-24 hours (includes time in formalin in processor; time may differ according to tissue type; e.g., surgical specimen, biopsy)
TISSUE PROCESSOR VARIABLES
Maintenance schedule: Manufacturer's recommendation or a validated deviation
Paraffin type: low melt <60°C
Total time in processor: 7.5-8 hours (forbid non-standard practices: e.g. "topping off with non-standard solutions)
GENERAL
Storage conditions are ambient (e.g., 20-25°C)
Document data for all recommendations.

3 Sample Preparation & Test Considerations[4,5]

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with the primary antibodies — the VENTANA CLDN18 (43-14A) IVD Assay, the LSBio PathPlus™ CLDN18 Antibody, and the Abcam Recombinant Anti-Claudin 18 antibody (43-14A).
It is recommended for IHC that tissue sections should be cut at approximately 4 µm thick and mounted on positively charged glass slides.
Before staining, the cut slides should be dried completely either at room temperature (air-dried) or offline baking (baked in oven) at 60°C for 60 minutes.
Slides should be stained promptly (within one week is recommended), as antigenicity of cut tissue sections may diminish over time and may be compromised due to environmental factors during extended storage.
It is recommended that positive and negative controls be run simultaneously with unknown specimens.
It is recommended that positive and negative run controls (i.e., system-level controls) be included as part of the unknown sample.

About the CLDN18.2 Expert Committee

The CLDN18.2 Expert Committee is comprised of some of the world's leading pathologists to develop and align best practices for how they test, interpret, and report CLDN18.2 expression.

The experts above have been contracted as paid advisors through Astellas Pharma Inc. and have received compensation for their time.

Matteo Fassan, MD, PhD
Padua, Italy

Takeshi Kuwata, MD, PhD
Tokyo, Japan

Kristina A. Matkowskyj, MD, PhD
Madison, WI, USA

Christoph Röcken, MD
Kiel, Germany

Josef H. Rüschoff, MD
Kassel, Germany

References: 1. College of American Pathologists. IHC assays—New evidence-based guideline for analytic validation (04-01-2004). https://documents.cap.org/documents/ihc-validation-webinar-handout.pdf. Accessed 03-30-2023. **2.** Fitzgibbons PL, Bradley LA, Fatheree LA, et al. Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2014;138(11):1432-1443. doi:10.5858/arpa.2013-0610-CP **3.** Compton CC, Robb JA, Anderson MW, et al. Preanalytics and precision pathology: pathology practices to enure molecular integrity of cancer patient biospecimens for precision medicine. *Arch Pathol Lab Med* 2019;143(11):1346-63. **4.** VENTANA CLDN18 (43-14A) assay [package insert]. **5.** Jasani B, Schildhaus HU, Taniere P, et al. Global ring study determining reproducibility and comparability of CLDN18 testing assays in gastric cancer. Poster presented at: ESMO Targeted Anticancer Therapies Congress; March 6-8, 2023; Paris, France.

G/GEJ = gastric/gastroesophageal

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