Construction and Validation of Alternative Biomarker Immunohistochemistry Assays on the Path to Companion Diagnostics

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INTRODUCTION

In vitro diagnostic IHC assays provide standardized biomarker testing, but dependence on a limited number of established providers may constrain platform flexibility, accessibility and study-specific customization. Alternative assays can expand testing options when they are developed and validated according to their intended use.

Here, we applied a structured, evidence-led workflow to develop 2 de novo cMET IHC assays across different staining platforms. The objective was not to reproduce every visual feature of a commercial reference assay, but to achieve robust analytical performance for the biological localization and scoring readout required by the study.

METHODS

Three c-MET IHC configurations were evaluated in 18 matched tumor samples: SP44 RxDx–VBU (reference), D1C2–BOND (alternative clone/platform), and SP44–DAKO AS (same clone/alternative platform). Samples represented a broad c-MET expression range: 5–100% for SP44 RxDx–VBU, 10–100% for D1C2–BOND, and 5–100% for SP44–DAKO AS. Prototype performance was assessed based on staining quality, expected localization, sensitivity, specificity, and signal-to-noise ratio. Derived mTPS and cmTPS were compared with the reference using Pearson and Spearman correlations, Lin's CCC, absolute-agreement ICC, and mean bias.

RESULTS INTERPRETATION

Representative staining across assay configurations

Platform-specific optimization generated robust c-MET staining across all assay configurations, with expected biological localization and interpretable tumor signal. Differences in staining appearance between clones and platforms reflected assay-specific characteristics, supporting evaluation against the intended localization and scoring strategy rather than visual similarity to the reference assay.

Preservation of sample-level biomarker readouts

Across 18 matched tumor samples, the developed assays preserved the overall dynamic range and sample ranking observed with the reference assay. Heatmap analysis showed similar patterns across high-, intermediate-, and low-expressing samples, indicating preservation of biologically relevant expression patterns across assay configurations.

Fit for purpose assay development workflow at CellCarta

1 | Define intended use
Biology • population of interest • clinical context • desired readout

2 | Assess feasibility use
Scientific rationale • technical suitability

3 | Evaluate options
Reagents (antibodies, platform specific reagents) • available platforms (Bond, Ventana, Dako)

4 | Optimize + verify staining
Protocol • localization • sensitivity • specificity

5 | Assess read out performance
Range • consistency • algorithm and scoring methodology testing

6 | Transition
Downstream validation guided by intended use

Results: representative staining

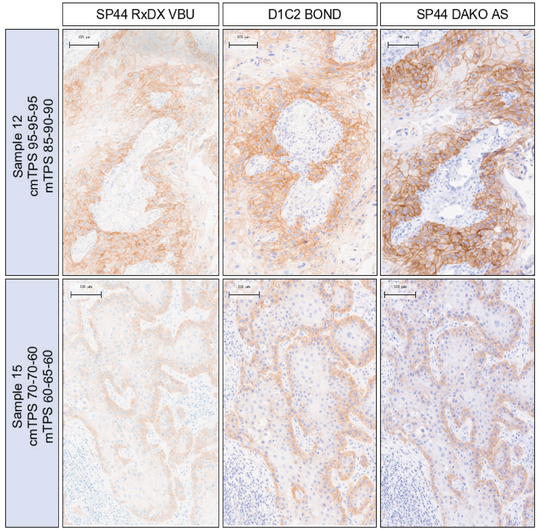


Figure 1:Representative c-MET IHC staining across assay configurations. Samples with high (Sample 12) and intermediate (Sample 15) c-MET expression are shown for SP44 RxDx VBU, D1C2 BOND, and SP44 DAKO AS. Corresponding cmTPS and mTPS scores are indicated for each assay. Scale bars = 100 µm.

Results: sample-level readouts

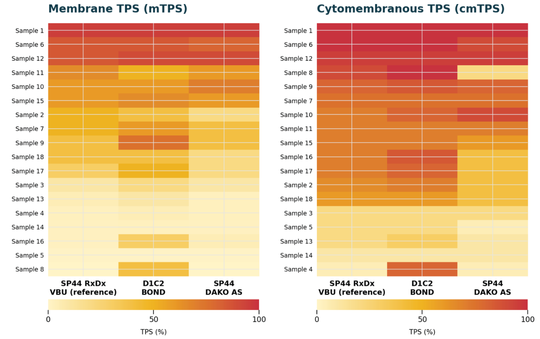


Figure 2: Heatmap comparison of mTPS and cmTPS across assay configurations across 18 samples. Derived mTPS and cmTPS scores are shown for 18 matched tumor samples evaluated with SP44 RxDx–VBU (reference), D1C2–BOND, and SP44–DAKO AS. Color intensity represents TPS (0–100%), allowing visualization of the dynamic range and sample-level differences across assay configurations.

Concordance with the reference assay

Strong agreement with the reference assay was observed across the evaluated endpoints. For membrane TPS, SP44-DAKO AS showed the highest agreement (CCC 0.955; ICC 0.957), while D1C2-BOND achieved CCC 0.867 and ICC 0.874. For cytomembranous TPS, CCC values were 0.805 and 0.774, respectively. Differences across endpoints highlight that assay agreement depends on the scoring readout, reinforcing the importance of evaluating performance according to intended use.

Key finding

Alternative clones and platforms can achieve strong analytical performance when developed using a fit-for-purpose strategy focused on the intended biological readout rather than visual similarity to a reference assay.

Results: concordance analysis

Assay Concordance vs SP44 RxDx - VBU

Continuous agreement analysis | 18 matched samples | Difference = comparator − reference

Endpoint	Comparator	n	Pearson r	Spearman	Lin CCC	ICC(A,1)	Mean bias
Derived mTPS %	SP44 - DAKO AS	18	0.961	0.974	0.955	0.957	-3.6
Derived mTPS %	D1C2 - BOND	18	0.897	0.850	0.867	0.874	7.6
Derived cmTPS %	D1C2 - BOND	18	0.835	0.831	0.805	0.813	7.8
Derived cmTPS %	SP44 - DAKO AS	18	0.823	0.837	0.774	0.783	-11.1

Figure 3: Agreement of mTPS and cmTPS between comparator assays and the SP44 RxDx–VBU reference assay. Across the four end-point–comparator combinations, Lin's concordance correlation coefficient (CCC) ranged from 0.774 to 0.955. For mTPS, agreement was highest for SP44–DAKO AS (CCC = 0.955; ICC = 0.957; mean bias = −3.6 percentage points), while D1C2–BOND showed a CCC of 0.867, ICC of 0.874, and mean bias of +7.6 percentage points. For cmTPS, CCC values were 0.805 for D1C2–BOND and 0.774 for SP44–DAKO AS. Agreement varied according to scoring endpoint, supporting evaluation of assay performance in the context of the intended readout rather than staining appearance alone.

CONCLUSIONS AND SIGNIFICANCE

- A structured, evidence-led workflow enabled the development of **high-quality cMET IHC assays across different staining platforms**.
- The data support a flexible model in which **assay construction is guided by the intended use**, relevant staining localization and scoring endpoint.
- This approach enables **customer-specific assay solutions** while maintaining the analytical rigor required for clinical-study workflows.

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