

# A META-ANALYSIS OF THE USE OF IHC TESTING IN METASTATIC DISEASE: THE NEED FOR INTEGRATION OF NEW TECHNOLOGIES

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## ABSTRACT

**Background:** Histologists use various panels of immunohistochemical (IHC) stains to identify the site of tissue of origin for metastatic tumors, particularly poorly or undifferentiated cancers of unknown or uncertain origin. While clinicians believe that immunostains contribute greatly to determining the probable primary site among three or more possibilities, objective evidence has not been convincingly presented. This meta-analysis reviews the objective evidence supporting this practice and summarizes the performance reported in five studies published between 1993 and 2007.

**Methods:** A literature search was conducted to identify all studies published since 1990 designed to quantify IHC performance in tissue determination. The literature search produced hundreds of IHC methodology articles, but only five met the pre-defined criteria; appropriately masked, including more than three tissue types and more than 50 specimens. Data from the five studies were separated into two subgroups for analysis: metastatic tumors only (n=368 specimens), and the blended studies which combined primary tumors and metastases (n=298 specimens).

**Results:** The meta-analysis found that IHCs provided the correct tissue identification for 82.3% (95% CI = 77.4 to 86.3%) of the blended primary and metastatic samples and 65.6% (95% CI=60.1 to 70.7%) of metastatic cancers. This difference is both clinically and statistically significant.

**Conclusions:** This meta-analysis confirms the clinical need for improved diagnostic methods in tissue of origin determination. It establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination. Finally, it underscores the importance of well-designed validation studies that are statistically sound, adequately sized, and appropriately masked. It is only through such validation that new, genomic diagnostic tests can establish an appropriate place in the practice of evidence-based medicine.

## BACKGROUND

Histopathologists today use various panels of immunohistochemical (IHC) stains to identify the site of origin for metastatic tumors, particularly poorly or undifferentiated cancers for which morphology alone provides limited diagnostic information. IHCs have proven to be highly useful tools for pair-wise differential diagnosis, to effectively rule out one or the other candidate tissue. However, choosing among three or more candidate tissues has proven to be a greater challenge given the limited specificity and sensitivity of IHCs available. While many pathologists believe that immunostains contribute greatly to determining the probable primary site among three or more possibilities, objective evidence has not been convincingly presented. This meta-analysis is intended to quantify the performance of IHC panels used in tissue determination through an examination of the published literature.

## MATERIALS AND METHODS

A literature review was performed using PubMed and the various search features it provides, including MeSH Terms, Limits, Related Articles, and text search within title and abstract fields. This series of searches identified hundreds of publications that describe immunohistochemistry methods for cancer tissue diagnosis, each of which includes a selection of tissues, a panel of IHCs to be evaluated for the purpose of differential diagnosis, and measurements of relative immunostaining. The majority of these publications involve simple, pair-wise discrimination of tissues, where IHC panels were used to rule out one or the other candidate tissue. A more challenging diagnostic problem is tumor identification when there are three or more candidate tissues.

Studies included in this meta-analysis were selected to meet the following criteria:

- The study must include three or more different tissue types or sites of origin.
- The study must include 50 or more specimens, either metastatic or primary tumors.
- The investigator (pathologist) must be blinded to the truth, or reference diagnosis and any additional clinical information, when interpreting the IHC panel results and drawing a conclusion.
- Reported performance must reflect the results of the blinded study.

Only five studies, published between 1993 and 2007 met all of these criteria (1-5).

### Tissues and IHC Panels

The studies included in this analysis evaluated the successful identification from among 3 to 7 different tissue types using a panel of 7 to 10 different IHCs. These differences are detailed in TABLE 1. Each investigator selected the IHCs to be included in the panel with complete, a priori knowledge of the tissue types to be included in the study. This a priori knowledge introduces favorable bias to the reported results.

### Sample Selection and the Reference Diagnosis

Specimens were selected, and the clinical truth or reference diagnosis was established based upon the pathology records in all five studies. In most of the studies, histologies were reviewed using H&E stains to select the most representative tissue block containing adequate volume of tumor. Only the *Park 2007* study went further, including an independent review of histologic slides by the author to confirm the reference diagnosis. There is no indication that this review altered the original reference diagnosis.

### Results Reporting

Several of the studies chose to group the reported results, e.g., as “gut” or “upper gastrointestinal tract”. These groupings artificially improve the reported performance of the IHC panel, with potential unfavorable impact on clinical interpretation and use of the diagnostic result. As such the groupings introduce favorable bias to the reported results.

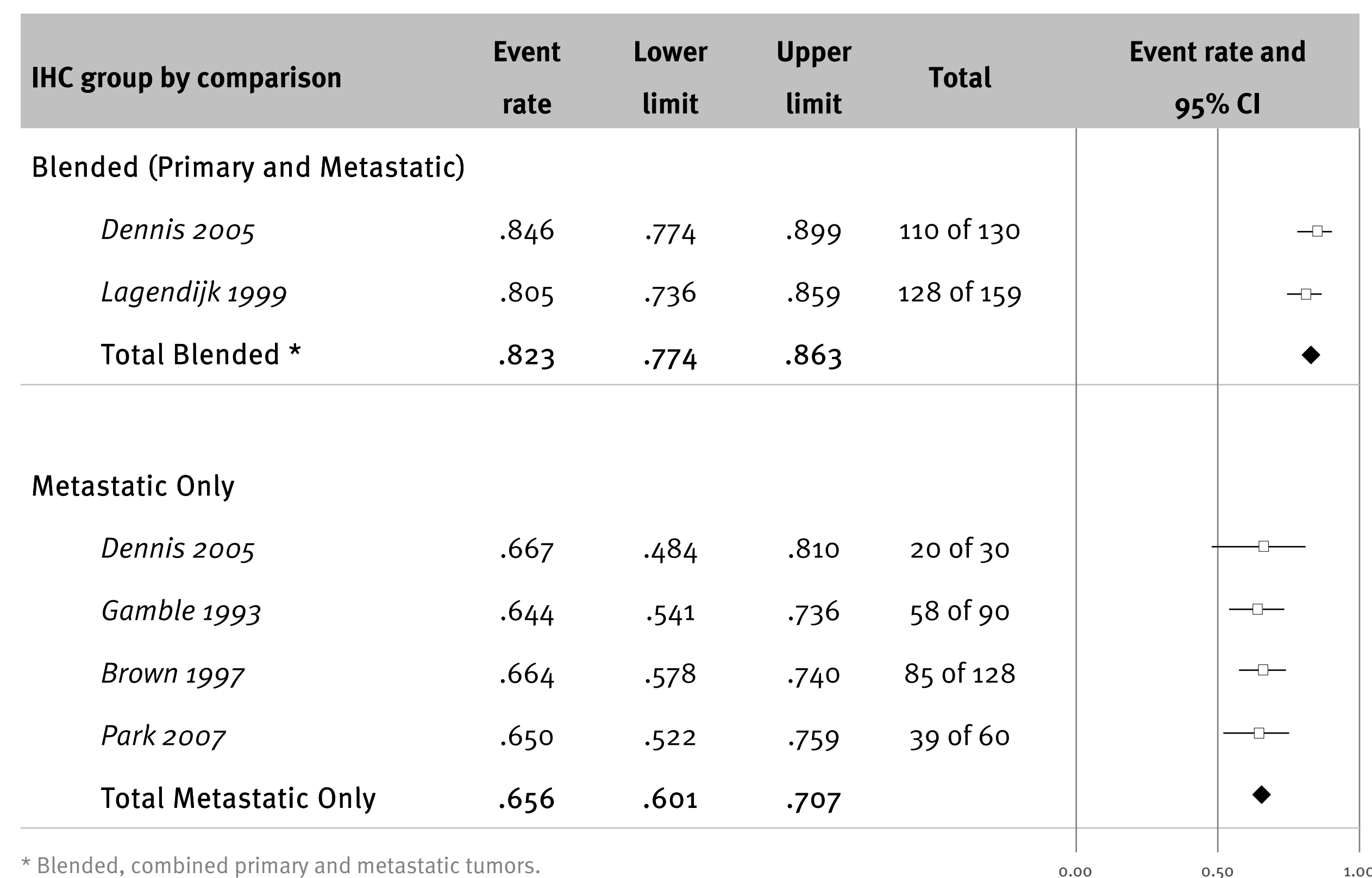
The *Brown 1997* study allowed the investigator to assign an indeterminate result for samples for which the stains were inadequate. The *Park 2007* study published higher accuracy results by omitting study samples that failed to stain from the reported results. To maintain consistency in reporting across all studies, these eight samples have been classified as indeterminate and included in the *Park 2007* study results and meta-analysis as shown in FIGURE 1.

## RESULTS

**TABLE 1. Various tissues and immunohistochemistry stains used in performance studies included in this review and meta-analysis**

Study	Tissues included	Immunohistochemistry stains used
Primary studies, appropriately blinded and included in the meta-analysis		
<i>Gamble 1993</i>	Breast, gut (colon, esophagus, stomach, small intestine), gynecological (ovarian, fallopian tube, endometrium), lung, other (pancreatic, renal, bladder and salivary gland)	NCRC-11, SM3, CEA228, CEA198, CA125, CA19-9, PSAP, PSA, thyroglobulin
<i>Brown 1997</i>	Breast, colon, lung, ovary, upper GI (stomach, pancreas and bile duct)	CEA, CA19-9, CA125, BCA225
<i>Lagendijk 1999</i>	Breast, colon, ovary	CK7, CK20, CA125, CEA, GCDFP-15, ER
<i>Dennis 2005</i>	Breast, colon, lung, ovary, pancreas, stomach, prostate	PSA, TTF1, GCDFP-15, CDX2, CK20, CK7, ER, mesothelin, CA125, lysozyme
<i>Park 2007</i>	Breast, colon, lung, ovary, pancreas, stomach, bile duct	CEA, TTF-1, CDX2, CK20, CK7, MUC2, MUC5AC, SMAD4, ER, GCDFP-15

**FIGURE 1. Meta-analysis of IHC performance results with subgrouping of the blended and metastatic studies**



**TABLE 2. IHC performance in tissue of origin identification: Metastatic Only**

IHC study	Tissues	Markers in panel	Training samples	Test samples	% Metastatic samples	Performance results		
						Correct (%)	Incorrect (%)	Indeterminate (%)
<i>Gamble 1993</i>	5	7	none	90	100%	58 (64%)	32 (36%)	0
<i>Brown 1997</i>	5	4	none	128	100%	85 (66%)	26 (20%)	17 (14%)
<i>Dennis 2005</i>	7	10	352	30	100%	20 (67%)	10 (33%)	0
<i>Park 2007</i>	7	10	314	60	100%	39 (65%)	8 (15%)	13 (22%)
Overall	5 – 7	4 – 10	0 – 352	30 – 128	100%	64 – 67%	15 – 36%	0 – 22%

**TABLE 3. IHC performance in tissue of origin site identification: Blended (primary and metastatic) studies**

IHC study	Tissues	Markers in panel	Training samples	Test samples	% Metastatic samples	Performance results		
						Correct (%)	Incorrect (%)	Indeterminate (%)
<i>Lagendijk 1999</i>	3	6	159	153	88%	128 (84%)	25 (16%)	N/A
<i>Dennis 2005</i>	7	10	352	130	23%	110 (85%)	20 (15%)	N/A
Overall	3 – 7	6 – 10	159 – 352	130 – 153	23 – 88%	84 – 85%	15 – 16%	N/A

## RESULTS (CONTINUED)

### Overall Results

#### Metastatic Only

Four studies for which the performance could be evaluated against metastatic samples alone reported that correct site identification was made in 64 to 67% of cases (1-3,5). Incorrect identifications made in these studies vary more broadly, from 15 to 36%, as two studies allowed indeterminate calls. FIGURE 1 details important characteristics of each of these studies and reports their performance.

Within each of these two subgroups, the individual studies show remarkably consistent performance. This is particularly noteworthy given the differences in the studies; different IHCs were included in each of the panels tested, different numbers and types of cancers were included in each study, and the studies were conducted by different laboratories over the course of 14 years.

#### Blended (Primary and Metastatic) Studies

Two studies (*Lagendijk 1999*, *Dennis 2005*) evaluated IHC performance using a combination, or blend, of metastatic and primary cancers as shown in TABLE 3. In these two studies, the site was successfully determined in 84 to 85% of cases, with incorrect determinations made for 15 to 16%. The primary cancers were predominately moderately differentiated.

### Meta-Analysis Results

A meta-analysis was performed to compare the results reported in these five published studies (1-5). Figure 1 presents the summary statistics and the forest plot. The analysis was performed in two subgroups, the metastatic tumor studies and the blended studies that included both primary and metastatic tumors.

#### Blended (Primary and Metastatic)

Meta-analysis of the blended studies, shown in Figure 1, suggests that IHC panels used for differential diagnosis provide an expected mean accuracy of 82.3% (95% CI = 77.4 to 86.3%) when applied to a combination of metastatic and primary cancers.

#### Metastatic Only

Meta-analysis of the metastatic subgroups, shown in Figure 1, suggests that IHC panels provide an expected mean accuracy of 65.6% (95% CI = 60.1 to 70.7%) when used for tissue determination of metastatic cancers.

## CONCLUSIONS

The meta-analysis of five studies published between 1993 and 2007 suggest that the blinded use of IHC panels selected for differential diagnosis of between 3 to 7 different tissues can identify the correct tissue of origin in 82.3% (95% CI = 77.4 to 86.3%) of cases involving primary or metastatic tumors, and 65.6% (95% CI = 60.1 to 70.7%) of metastatic tumors. This difference is both statistically significant and clinically significant. The results confirm that IHC panels alone perform poorly in determining tissue of origin in metastatic tumors.

Overall, it is important to recognize that tissue determination in actual clinical practice uses a combination of IHC results and clinical information including biopsy site, patient gender, age and clinical history. This clinical information was masked in all of the studies included in this analysis. The masking introduces unfavorable bias to the reported results relative to more typical clinical use. However, the reported performance may better represent what can be expected in those cases for which clinical information is unable to provide significant additional information. This is reported to be true of poorly or undifferentiated tumors found in unexpected locations, including carcinomas of unknown primary.

This meta-analysis establishes minimum performance requirements for any new diagnostic test intended to aid the pathologist and oncologist in tissue of origin determination. It underscores the importance of well-designed validation studies that are statistically sound, adequately sized, and appropriately masked. It is only through such validation that a new, genomic diagnostic test can establish an appropriate role in the practice of evidence-based medicine.

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