**Instructional Statement for p40 (BC28) Abstract Summary Sheet**

p40 (BC28) is a sensitive and specific antibody for the detection of p40 (ΔNp63) protein. This antibody in conjunction with a panel of other key markers in our portfolio (TTF-1, CK 5/6, Napsin A) can provide a reliable method for differentiating pulmonary adenocarcinoma from squamous cell carcinoma.

Multiple studies indicate that p40 has comparable sensitivity but higher specificity in non-small cell lung carcinoma as compared to p63. The use of p40 is recommended instead of p63 for sub-classifying non-small cell lung cancer to avoid falsely interpreting p63+ adenocarcinoma as belonging to the category of squamous cell carcinoma.

This document highlights available publications that demonstrate the utility and performance of p40 in various tissues.

**Lung, Bladder, Skin, Breast, Prostate, Head and Neck Cancers**

Publication: Tacha, David PhD; Ryan Bremer, PhD; Thomas Haas, DO; Weiman Qi, PhD, MD. An Immunohistochemical Analysis of a Newly Developed, Mouse Monoclonal p40 (BC28) Antibody in Lung, Bladder, Skin, Breast, Prostate, and Head and Neck Cancers. Arch Pathol Lab Med—Vol 138, October 2014.

The major goal of this study was to evaluate the specificity and sensitivity of p40 (BC28) mouse monoclonal primary antibody in normal and neoplastic tissues with emphasis on lung cancer. The p40 (BC28) antibody was also evaluated in breast, bladder, skin, prostate, and head and neck cancers. The staining characteristics of p40 (BC28), p63 (4A4) and p40 (polyclonal) antibodies were compared in different types of lung cancers. In addition, p40 (BC28) antibody was evaluated in benign prostate glands and in prostatic intraepithelial neoplasia and compared with the p63 (4A4) antibody.

The results showed superiority of p40 (BC28) compared to p63 (4A4) in the differentiation of lung tumors. The data indicate that p40 (BC28) has sensitivity equal to that of p63 (4A4) in lung squamous cell carcinoma, however fewer lung adenocarcinoma cases were p40 positive, supporting a claim of increased specificity of p40 (BC28) over p63 (4A4). Furthermore, notably superior staining was observed with p40 (BC28) compared to p40 (polyclonal) in lung cancer cases, where p40 (polyclonal) displayed nonspecific, cytoplasmic staining. In addition, the data in this study provide the evidence for the use of p40 (BC28) as a high-quality antibody for determining squamous cell carcinoma in lung, skin, head and neck and also in urothelial carcinomas. p40 (BC28) displayed a cleaner and more intense staining pattern when compared to p63 in basal cells in prostate tissue.

**Lung – Squamous Cell Carcinoma (and Large Cell Lymphoma)**


The purpose of this study was to compare the specificity and sensitivity of the p40 (polyclonal) antibody to that of a p63 (4A4) antibody in different lung tumors and large cell lymphoma. Overall, 470 cases including 81 lung squamous cell carcinomas, 237 lung adenocarcinomas and 152 large cell lymphomas were evaluated in this study.

The data indicate that although the sensitivity of p40 in squamous cell carcinoma is comparable to p63 (4A4) antibody, the specificity of p40 is significantly better than p63 (4A4). These results provide support for the routine use of p40 instead of p63 for the diagnosis of pulmonary squamous cell carcinoma.
**Lung – Non-Small Cell Carcinoma**
This study compared the staining characteristics of p40 (polyclonal), p63 (4A4) and TTF-1 (8G7G3/1) in 150 lung adenocarcinomas and 50 squamous cell carcinomas.

**The overall conclusion of this study is that p40 (polyclonal) is a sensitive and specific marker for squamous cell carcinoma. This marker may be a better solution than p63 (4A4) for distinguishing between adenocarcinoma and squamous cell carcinoma of the lung.**

**Lung – Non-Small Cell Carcinoma**

The aim of this study was to evaluate a panel of different IHC markers in subtyping poorly differentiated NSCLC. There were 48 cases included in the study that could not be classified by H&E alone. The markers evaluated in this study included p40 (∆Np63), CK 5/6 (D5/16B4), TTF-1 (8G7G3/1), Napsin A (MRQ–60), p63 (4A4), CK 7 (OV-TL12/30), CK 8/18 (5D3) and Keratin 34βE12.

The study showed that a panel including p40 (polyclonal), CK 5/6 (D5/16B4), TTF-1 (8G7G3/1), and Napsin A (MRQ–60) correctly classified 39 of 48 cases (81%) of poorly differentiated NSCLC. Nine tumors could not be further classified. The inclusion of p40 (polyclonal) as a substitute for p63 (4A4) in this panel was based on findings that p40 provided a higher specificity than p63 for lung adenocarcinoma and equal sensitivity for lung squamous cell carcinoma. CK 7, CK 8/18 and 34βE12 were excluded from the panel due to low specificity of CK 7 and CK 8/18 for adenocarcinoma and 34βE12 for SCC. In conclusion, the proposed IHC panel for lung cancer classification includes the following markers: p40, CK 5/6, TTF-1, and Napsin A.

**Lung – Non-Small Cell Lung Carcinoma**
Publication: Luisella Righi, MD, PhD, Tiziana Vavalà, MD, Ida Rapa, BSc, Simona Vatrano, BSc, Jessica Giorcelli, Giulio Rossi, MD, Enrica Capelletto, MD, Silvia Novello, MD, Giorgio V. Scagliotti, MD, and Mauro Papotti, MD. Impact of Non–Small Cell Lung Cancer–Not Otherwise Specified Immunophenotyping on Treatment Outcome. J Thorac Oncol. 2014;9:1540–1546.

IHC is the most commonly used approach to classify histological lung tumors. This study evaluated the benefit of immunohistochemical subtyping for the clinical outcome in 224 cases of NSCLC—not otherwise specified (NOS) tumors. These cases were classified as adenocarcinomas and NSCLC-NOS based on morphology. The NSCLC-NOS cases were further evaluated using a panel of four markers, including p40 (BC28), TTF-1 (8G7G3/1), Napsin–A (TMU-Ad02) and Desmocollin–3 (DSC3).

This study indicates that immunophenotyping of poorly differentiated NSCLC using p40 (BC28), TTF-1 (8G7G3/1), Napsin–A (TMU-Ad02) and Desmocollin–3 (DSC3) is beneficial for patients in terms of therapeutic strategy. NSCLC-NOS cases with an unclear morphology but an immunophenotype that suggests ADC have similar response to chemotherapy and outcome comparable to that of ADC cases. Overall, this study supports the use of an IHC panel in combination with morphology to classify poorly differentiated tumor subtypes, which may consequently lead to appropriate therapeutic decisions.
**Lung – Squamous Cell Carcinoma**


The major goal of this study was to evaluate p40 expression and compare it with previously reported studies evaluating the expression of p63, CK 5/6, Sox2, and Desmocollin-3 in 539 primary lung carcinomas and 41 malignant mesotheliomas.

The results presented in this paper showed high sensitivity and specificity of p40 (polyclonal) in distinguishing squamous cell carcinoma (SQC) from adenocarcinoma (ADC), neuroendocrine carcinomas (NEC) or malignant mesothelioma. The p40 is considered to be the “best marker” for differentiating SQC from non-SQC. Similar to several previously published studies, this paper suggests that p40 should replace p63 as a routine SQC marker.

**Lung – Small Cell Carcinoma**


The staining characteristics of p40 (polyclonal), p63 (4A4), and Keratin 34βE12 in 34 small cell lung carcinomas (27 small biopsies, 7 large samples) were evaluated in this paper.

All small cell lung carcinoma cases were p40 negative, however a positive p63 staining was observed in 12 (44.4%) of the 27 biopsy samples. These results provide evidence for the routine use of p40 instead of p63 in the differentiation of lung tumors in small biopsy samples.

**Prostate – Basal Cells**


This study evaluated the diagnostic value of p40 (polyclonal) as a basal cell marker in prostate tissues. As the basal cells are absent from invasive prostate adenocarcinoma, p40 expression can be used to differentiate between benign and malignant glands. In this study the staining performance of p40 (polyclonal) and p63 (4A4) was compared in a total of 640 normal and neoplastic prostate tissues.

The results of this study demonstrated that p40 is an excellent marker for basal cells, which is more specific than p63. A higher rate of aberrant staining of tumor cells was observed in tissues stained with p63 (4A4). However, a higher rate of cytoplasmic staining was detected in tissues stained with p40 (polyclonal). This would limit the usefulness of p40 in antibody cocktails, although, this particular issue could potentially be resolved with the use of a p40 monoclonal antibody such as BC28.

Items herein are the opinion of Ventana Medical Systems, Inc.
**Prostate – Basal Cells**


The major aim of this study was to evaluate the staining performance of p40 (polyclonal) and compare it to Keratin 34betaE12 in small biopsies from 68 patients with prostate atypical glandular proliferation.

**The results of these experiments provide support for the use of p40 in the diagnosis of suspicious prostate glands.** It demonstrated a close correlation between staining using 34betaE12 and p40 (polyclonal) in basal cells. Although morphological assessment is the primary tool, IHC testing should be considered as an aid to the final diagnosis. The study indicates that IHC using p40 may be highly useful for the identification of basal cells of benign prostate glands, especially in difficult cases.

**Salivary Glands – Polymorphous low grade adenocarcinoma (PLGA)**

Publication: Rooper, Lisa, Rajni Sharma, Justin A. Bishop. Polymorphous Low Grade Adenocarcinoma has a Consistent p63+/p40- Immunophenotype that Helps Distinguish it from Adenoid Cystic Carcinoma and Cellular Pleomorphic Adenoma. Head and Neck Pathol. 2014. DOI 10.1007/s12105-014-0554-4.

This study evaluated the utility of p40 (BC28) and p63 (4A4) in identifying tumors of minor salivary glands using tissues from 11 cases of polymorphous low grade adenocarcinoma (PLGA), 101 cases of adenoid cystic carcinomas and 31 cases of pleomorphic adenomas.

**The results of these experiments demonstrated the usefulness of p40 (BC28) in “distinguishing salivary gland tumors with true myoepithelial differentiation from those showing nonspecific p63 expression.”** The study also highlighted the utility of using p40 (BC28) and p63 (4A4) in a two antibody panel to reliably distinguish between PLGA from both adenoid cystic carcinoma and pleomorphic adenoma as PLGA cases were consistently p63+/p40-.