(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 April 2014 (03.04.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/052672 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/30* (2006.01)
C07K 16/28 (2006.01)

(21) International Application Number:
PCT/US2013/062043

(22) International Filing Date:
26 September 2013 (26.09.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/706,312 27 September 2012 (27.09.2012) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: ANTI-UROPLAKIN II ANTIBODIES SYSTEMS AND METHODS

(57) Abstract: The present invention is related to the anti-Uroplakin II antibodies, kits, cocktails, and use of anti-Uroplakin II antibodies for detection of cancer.

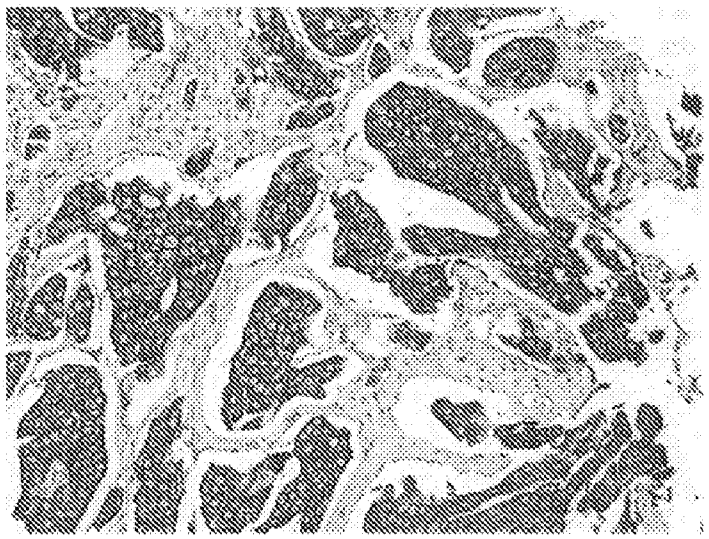

FIG. 1

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— *with sequence listing part of description (Rule 5.2(a))*

ANTI-UROPLAKIN II ANTIBODIES SYSTEMS AND METHODS

This application is an International PCT Patent Application claiming priority to and the benefit of U.S. Provisional Application No. 61/706,312 filed September 27, 2012 hereby incorporated by reference herein in its entirety.

TECHNICAL FIELD

This invention relates to novel anti-Uroplakin II antibodies, compositions, cocktails, and kits comprising the antibodies and methods for using the antibodies.

BACKGROUND OF THE INVENTION

Microscopic examination of tissue samples, particularly those obtained by biopsy, is a common method for diagnosis of disease. In particular, immunohistochemistry (IHC), a technique in which specific antibodies are used to detect expression of specific proteins in the tissue sample, is a valuable tool for diagnosis, particularly for the detection and diagnosis of cancer.

Uroplakins ("UP" or "Ups") comprise a group of 4 transmembrane proteins (UPs Ia, Ib, II, and III) expressed in the luminal surface of normal urothelial superficial (umbrella) cells, which are specific differentiation products of urothelial cells. Uroplakin II ("UP II") may be a 15 kDa protein component of the urothelial plaques which may enhance the permeability barrier of the urothelium. The expression of UP II may be aberrant in urinary bladder transitional cell carcinoma ("TCC") of the bladder and thus may make it a useful marker for the diagnosis of cancer. The Wu *et al.* reference includes discussion of UP II mRNA as a promising diagnostic marker for bladder cancer and perhaps even micrometastases of bladder cancer in the pelvic lymph nodes (*see* article, "Uroplakin II as a promising marker for molecular diagnosis of nodal metastases from bladder cancer: comparison with cytokeratin 20." Wu X, Kakehi Y, Zeng Y, Taoka R, Tsunemori H, Inui M. J Urol. 2005 Dec;174(6):2138-42, hereby incorporated by reference herein.) UP II mRNA was detected in 19 of 19 (100%) and 15 of 16 (93.8%) bladder tumor tissue specimens and pelvic lymph node samples with metastasis, respectively. On the other hand, UP II mRNA was detected in only 6 cases out of 66 (10%) pelvic lymph node samples without metastasis. Therefore, positive expression of UP II mRNA may indicate nodal metastases from bladder cancer. It may be important to determine the nodal metastases in patients with bladder

cancer after radical cystectomy as this subpopulation of patients may urgently need postoperative chemotherapy to survive. The authors conclude that the detection of UP II mRNA may improve clinical outcome following radical cystectomy perhaps by providing helpful information in the diagnosis and management of TCC. It is desirable, therefore, for development of an anti-UP II antibody for detection of UP-II protein expression in the tissues of patients such as with TCC or the like.

Studies have shown UP II mRNA to be expressed in bladder tissues and peripheral blood of patients with primary and metastatic TCCs, perhaps suggesting its potential role as a biomarker for urothelial carcinomas. The clinical usefulness of UP II may have been recognized from these studies perhaps solely based on its mRNA data. Additional investigations characterizing the protein localization of UPII in TCC may be warranted, especially when the protein molecules may be more stable than mRNA molecules. One study employed a pan-UP antibody which may have reacted with all UPIb, UPII, and even UPIII isoforms perhaps to demonstrate the persistent expression of UP in advanced urothelial carcinomas; however, the specific UP II protein level could not be determined using this pan-UP antibody. (*See*, Persistent Uroplakin Expression in Advanced Urothelial Carcinomas: Implications in Urothelial Tumor Progression and Clinical Outcome. Hong-Ying Huang, Shahrokh F. Shariat, Tung-Tien Sun, Herbert Lepor, Ellen Shapiro, Jer-Tsong Hsieh, Raheela Ashfaq, Yair Lotan, and Xue-Ru Wu, Hum Pathol. 2007 November; 38(11): 1703–1713, hereby incorporated by reference herein). Little may be known about the protein expression of UP II in urothelial cancer, possibly due to the absence of a specific anti-UP II antibody.

A clear need exists for a sensitive and even specific anti-Uroplakin II antibody for use in cancer diagnosis. Anti-UP III antibodies have been previously developed as markers for carcinoma of urothelial origin. Here the present invention provides an anti-UP II antibody [clone BC21] which may be highly specific and may even be more sensitive than the anti-UP III antibody [clone BC17]. In cases of TCC, an example of the present invention provides an anti-UP II antibody that exhibited an increased sensitivity (about 46/59, about 78%) compared to the anti-UP III antibody (about 33/59, about 56%). Perhaps in addition to its stronger staining profile, the anti-UP II antibody [BC21] may exhibit a wider localization pattern compared to anti-UP III antibody. This could be due to the

superior sensitivity of the anti-UP II antibody or perhaps even the two isoforms may have distinct roles in the formation of urothelial plaques. The difference in their function may not be fully known; however, mice lacking the UP II gene showed no urothelial plaque formation while mice lacking the UP III gene may still retain small urothelial plaques. If UP II and UP III indeed exhibit non-overlapping functions, determination of either isoform may not be sufficient for an effective diagnosis of TCC. Therefore, an anti-UP II antibody may be needed for a more complete coverage of protein expression of UP isoforms.

The development of an anti-UP II antibody may aid in the diagnosis of primary and even metastatic TCCs, may aid in the verification of UP II mRNA expression perhaps in previous clinical studies, and may even aid in distinguishing a protein expression of UP II versus UP III. New anti-Uroplakin II antibodies such as anti-Uroplakin II antibody [BC21] with perhaps increased staining sensitivity, and perhaps even while preserving equal or even superior staining specificity such as compared to anti-Uroplakin III antibody [BC17], have been provided in the present invention.

DISCLOSURE OF THE INVENTION

General embodiments of the present invention may include monoclonal antibodies for recognizing UP II, methods for their preparation, use in immunohistochemistry, or the like. In embodiments, anti-UPII antibody clones such as the anti-UP II antibody clone BC21 can be obtained by immunizing Balb/C mice with a recombinant human UP II protein corresponding to amino acids 26-155, obtained by *E. coli* expression. The UP II proteins may be injected into the BALB/c mice, with an adjuvant, via intraperitoneal injections, perhaps about 5 times at about three week intervals. The immune reactivity to UP II may be assessed by direct ELISA on recombinant UP II protein. Mice with the highest titer may be chosen for developing hybridomas by cell fusion. A hybridoma clone demonstrating the best reactivity to UP II on human tissues may be chosen and may be designated as BC21. The BC21 clone may be tested for isotype and may be identified as a mouse IgG1/kappa. The BC21 antibody may be produced by large scale tissue culture of the hybridoma cells and by ascites in BALB/c mice. The supernatant and antibody ascites may be collected and the antibody may be purified by Protein A affinity column. BC21 demonstrated specific reactivity to human UP II protein by ELISA, Western blotting, and even human tissues.

Anti-UPII antibodies such as the mouse monoclonal anti-UP II antibody BC21 may be useful for the detection of UP II in tissue samples, perhaps with several significant, but unexpected advantages over currently known anti-UP III antibodies. When used in traditional immunohistochemistry procedures, anti-UPII antibodies such as the mouse anti-UP II antibody BC21 may result in membrane or cytoplasmic staining of UP II with a specificity perhaps similar to that of known anti-UP III antibodies. However, anti-UPII antibodies such as BC21 may exhibit increased sensitivity, perhaps as compared to past anti-UP III antibodies, which may offer significant improvements. With anti-UPII antibodies such as BC21, analysis of the sample may be simplified and UP II expression in tumor cells may be readily identifiable, allowing diagnosis in cases that may otherwise be difficult, or not even possible, to diagnose.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 2 shows an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 1.

Figure 3 shows an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 2).

Figure 4 shows an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 3.

Figure 5 shows an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 6 shows an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 5.

Figure 7 shows an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 8 shows an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 7.

Figures 9A and 9B show the cross-reactivity of BC21 and BC17 antibodies with Uroplakin II protein and Uroplakin III protein by Western blot.

Figure 10 shows an example of a cocktail of UPII + UPIII staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic.

Figure 11 shows an example of a cocktail of UPII + GATA3 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 12 shows an example of a cocktail of UPII + GATA3 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 13 shows an example of a cocktail of UPII + UPIII + GATA3 staining urothelial carcinoma. Staining of UPII and UPIII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 14 shows an example of a cocktail of UPII + PAX8 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 15 shows an example of a cocktail of UPII + PAX8 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 16 shows an example of a cocktail of UPII + PAX8 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 17 shows an example of a cocktail of UPII + PAX8 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 18 shows an example of a cocktail of UPII + PAX8 + PSA staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 and PSA (nuclear and cytoplasmic, respectively; brown) may be reduced or perhaps absent in this sample.

Figure 19 shows an example of a cocktail of UPII + PAX8 + PSA staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) and PSA (cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 20 shows an example of a cocktail of UPII + PAX8 + PSA staining prostate cancer. Staining of PSA (brown) is cytoplasmic. Staining of UPII (membranous and cytoplasmic, red) and PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 21 shows an example of a cocktail of UPII + PAX8 + PSA staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of PAX8 and PSA (nuclear and cytoplasmic, respectively; red) may be reduced or perhaps absent in this sample.

Figure 22 shows an example of a cocktail of UPII + PAX8 + PSA staining renal cell carcinoma. Staining of PAX8 (red) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and PSA (cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 23 shows an example of a cocktail of UPII + PAX8 + PSA staining prostate cancer. Staining of PSA (red) is cytoplasmic. Staining of UPII (membranous and cytoplasmic, brown) and PAX8 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 24 shows an example of a cocktail of UPII + NKX3.1 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of NKX3.1 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 25 shows an example of a cocktail of UPII + NKX3.1 staining prostate cancer. Staining of NKX3.1 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 26 shows an example of a cocktail of UPII + PAX8 + NKX3.1 staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) and NKX3.1 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 27 shows an example of a cocktail of UPII + PAX8 + NKX3.1 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and NKX3.1 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 28 shows an example of a cocktail of UPII + PAX8 + NKX3.1 staining prostate cancer. Staining of NKX3.1 (red) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 29 shows an example of a cocktail of UPII + p63 staining urothelial carcinoma. Staining of p63 (brown) is nuclear. Staining of UPII (red) is membranous and cytoplasmic.

Figure 30 shows an example of a cocktail of UPII + p63 staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of p63 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 31 shows an example of a cocktail of UPII + p63 staining prostatic intraepithelial neoplasia (PIN). Staining of p63 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 32 shows an example of a cocktail of UPII + p63 staining normal prostate. Staining of p63 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 33 shows an example of a cocktail of UPII + p63 staining urothelial carcinoma. Staining of p40 (brown) is nuclear. Staining of UPII (red) is membranous and cytoplasmic.

Figure 34 shows an example of a schematic summary of a kit in accordance with various embodiments of the present invention.

Figure 35 shows an example of a schematic summary of an immunoassay method in accordance with various embodiments of the present invention.

Figure 36 is a color version of Figure 1 showing an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 37is a color version of Figure 2 showing an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 36.

Figure 38 is a color version of Figure 3 showing an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 2).

Figure 39 is a color version of Figure 4 showing an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 38.

Figure 40 is a color version of Figure 5 showing an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 41 is a color version of Figure 6 showing an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 40.

Figure 42 is a color version of Figure 7 showing an example of anti-UP II antibody [BC21] staining on bladder TCC tissue (grade 3).

Figure 43 is a color version of Figure 8 showing an example of anti-UP III antibody [BC17] staining on a serial section of the same bladder TCC tissue of Figure 42.

Figures 44A and 44B is a color version of Figures 9A and 9B showing the cross-reactivity of BC21 and BC17 antibodies with Uroplakin II protein and Uroplakin III protein by Western blot.

Figure 45 is a color version of Figure 10 showing an example of a cocktail of UPII + UPIII staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic.

Figure 46 is a color version of Figure 11 showing an example of a cocktail of UPII + GATA3 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 47 is a color version of Figure 12 showing an example of a cocktail of UPII + GATA3 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 48 is a color version of Figure 13 showing an example of a cocktail of UPII + UPIII + GATA3 staining urothelial carcinoma. Staining of UPII and UPIII (red) is membranous and cytoplasmic. Staining of GATA3 (brown) is nuclear.

Figure 49 is a color version of Figure 14 showing an example of a cocktail of UPII + PAX8 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 50 is a color version of Figure 15 showing an example of a cocktail of UPII + PAX8 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 51 is a color version of Figure 16 showing an example of a cocktail of UPII + PAX8 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 52 is a color version of Figure 17 showing an example of a cocktail of UPII + PAX8 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 53 is a color version of Figure 18 showing an example of a cocktail of UPII + PAX8 + PSA staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of PAX8 and PSA (nuclear and cytoplasmic, respectively; brown) may be reduced or perhaps absent in this sample.

Figure 54 is a color version of Figure 19 showing an example of a cocktail of UPII + PAX8 + PSA staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) and PSA (cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 55 is a color version of Figure 20 showing an example of a cocktail of UPII + PAX8 + PSA staining prostate cancer. Staining of PSA (brown) is cytoplasmic. Staining of UPII (membranous and cytoplasmic, red) and PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 56 is a color version of Figure 21 showing an example of a cocktail of UPII + PAX8 + PSA staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of PAX8 and PSA (nuclear and cytoplasmic, respectively; red) may be reduced or perhaps absent in this sample.

Figure 57 is a color version of Figure 22 showing an example of a cocktail of UPII + PAX8 + PSA staining renal cell carcinoma. Staining of PAX8 (red) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and PSA (cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 58 is a color version of Figure 23 showing an example of a cocktail of UPII + PAX8 + PSA staining prostate cancer. Staining of PSA (red) is cytoplasmic. Staining of UPII (membranous and cytoplasmic, brown) and PAX8 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 59 is a color version of Figure 24 showing an example of a cocktail of UPII + NKX3.1 staining urothelial carcinoma. Staining of UPII (red) is membranous and cytoplasmic. Staining of NKX3.1 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 60 is a color version of Figure 25 showing an example of a cocktail of UPII + NKX3.1 staining prostate cancer. Staining of NKX3.1 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, red) may be reduced or perhaps absent in this sample.

Figure 61 is a color version of Figure 26 showing an example of a cocktail of UPII + PAX8 + NKX3.1 staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of PAX8 (nuclear, brown) and NKX3.1 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 62 is a color version of Figure 27 showing an example of a cocktail of UPII + PAX8 + NKX3.1 staining renal cell carcinoma. Staining of PAX8 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and NKX3.1 (nuclear, red) may be reduced or perhaps absent in this sample.

Figure 63 is a color version of Figure 28 showing an example of a cocktail of UPII + PAX8 + NKX3.1 staining prostate cancer. Staining of NKX3.1 (red) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) and PAX8 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 64 is a color version of Figure 29 showing an example of a cocktail of UPII + p63 staining urothelial carcinoma. Staining of p63 (brown) is nuclear. Staining of UPII (red) is membranous and cytoplasmic.

Figure 65 is a color version of Figure 30 showing an example of a cocktail of UPII + p63 staining urothelial carcinoma. Staining of UPII (brown) is membranous and cytoplasmic. Staining of p63 (nuclear, brown) may be reduced or perhaps absent in this sample.

Figure 66 is a color version of Figure 31 showing an example of a cocktail of UPII + p63 staining prostatic intraepithelial neoplasia (PIN). Staining of p63 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 67 is a color version of Figure 32 showing an example of a cocktail of UPII + p63 staining normal prostate. Staining of p63 (brown) is nuclear. Staining of UPII (membranous and cytoplasmic, brown) may be reduced or perhaps absent in this sample.

Figure 68 is a color version of Figure 33 showing an example of a cocktail of UPII + p40 staining urothelial carcinoma. Staining of p40 (brown) is nuclear. Staining of UPII (red) is membranous and cytoplasmic.

MODE(S) FOR CARRYING OUT THE INVENTION

As may be understood from the earlier discussion, the present invention includes a variety of aspects, which may be combined in different ways. The following descriptions are provided to list elements and describe some of the embodiments of the present invention. These elements are listed with initial embodiments, however it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described systems, techniques, and applications. Further, this description should be understood to support and encompass descriptions and claims of all the various embodiments, systems, techniques, methods, devices, and applications with any number of the disclosed elements, with each element alone, and also with any and all various permutations and combinations of all elements in this or any subsequent application.

Embodiments of the present invention may provide antibodies and methods thereof that specifically bind to UP II and may be used for the detection of UP II in the diagnosis for several types of cancers. An antibody may be an antibody fragment, a mouse monoclonal antibody, a chimeric antibody, a humanized monoclonal antibody, a human monoclonal antibody, an antibody with a label attached or even conjugated therewith or with a fragment thereof, an antibody labeled with a detectable signal or stain, an antibody labeled with a toxin, or the like. A label may include but is not limited to radioactive element, magnetic particles, radioisotope, fluorescent dye, enzyme, toxin, signal, stain, detection enzymes, horseradish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase, chromogens, Fast Red, 3,3'-diaminobenzidine, 3-amino-9-ethylcarbazole, 5-bromo-4-chloro-3-indolyl phosphate, 3,3′,5,5′-tetramethylbenzidine, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, any

combination thereof, or the like. Systems and methods of the present invention may relate to the antibody or its antigen binding portion capable of binding to UP II.

Embodiments of the present invention may provide monoclonal antibodies and methods thereof that specifically bind to UP II and may be used for the detection of UP II in the diagnosis for several types of cancers. The monoclonal antibody may be an antibody fragment, a mouse monoclonal antibody, a rabbit monoclonal antibody, a chimeric antibody, a humanized monoclonal antibody, a human monoclonal antibody, an antibody labeled with a detectable signal or stain, an antibody labeled with a toxin, or the like. Systems and methods of the present invention may relate to the monoclonal antibody or its antigen binding portion capable of binding to UP II.

Mouse monoclonal antibodies may be commonly used in immunoassay methods to identify specific analytes, including as primary antibodies in immunohistochemistry procedures. Mouse monoclonal antibodies specific for the protein target of interest can be produced using generally known procedures. Generally, exposing a mouse to the antigen of interest (e.g. a peptide fragment of the desired target or the full-length protein target) may induce an immune response in which the mouse generates multiple antibodies that bind the antigen, each of which may be produced by a particular B-cell. These B-cells may be isolated from the mouse spleen and the antibodies produced may be evaluated for their suitability as primary antibodies in IHC. After selecting the optimal antibody, the associated B-cell may be fused with a tumor cell using known procedures, perhaps resulting in a hybridoma, a new cell line that can endlessly replicate and may continuously produce the desired antibody.

Monoclonal antibodies may be preferred over polyclonal antibodies for several reasons. In particular, monoclonal antibodies may be derived from a single B-cell and as such may recognize a single epitope, perhaps resulting in greater specificity. Monoclonal antibodies may also be conveniently and reproducibly generated in cell culture, perhaps resulting in a constant supply of the desired antibody. Of course, polyclonal antibodies may be used in some embodiments.

Anti-UPII antibodies such as a mouse monoclonal anti-UP II antibody BC21 may be produced using these general procedures and may be evaluated by immunohistochemistry

for sensitivity and specificity on a variety of normal and neoplastic tissues, perhaps particularly in comparison to the previously known anti-UP III antibody [BC17].

Example of UPII protein expression: A UPII recombinant protein from amino acid sequence 26 to 155 may be cloned and expressed from *E. coli*. Briefly, UPII cDNA may be cloned and purified. The UPII cDNA may be digested by restriction enzymes and ligated into the pET30a-GST vector. BL21 cells may be transformed with the construct. The colonies expressing the correct size of recombinant protein may be selected and sequenced. A further scale up production may be performed by culturing the *E. coli* in LB media containing 0.5mM IPTG. The final UPII recombinant protein may be purified and analyzed by SDS-PAGE.

Example of Host Immunization: Female BALB/c (about 6 to about 8 weeks old) mice may be immunized intraperitoneally (i.p.) with about 100µg human UPII protein per mouse in complete Freund's adjuvant. About three weeks later, the mice may be boosted with another 100µg human UPII per mouse in incomplete Freund's adjuvant about 4 more times in about 3 week intervals. Mice may be bled from the tails, and sera may be collected and stored at -20°C for later analysis of antibody titers by enzyme-linked immunosorbent assay (ELISA).

Example of Hybridomas: Hybridomas producing antibodies to UPII may be generated by standard techniques from splenocytes of UPII-immunized BALB/c mice. For example, splenocytes from UPII-immunized mice may be fused to P3-X63-Ag 8.653 myeloma cells (non-secreting myeloma derived from SP2/0 Balb/c myeloma cells) by incubation with about 50% polyethylene glycol at a ratio of about 4:1. Following incubation, cells may be pelleted by centrifugation perhaps at about 300 × g for about 10 minutes, washed in about 25 ml of PBS, recentrifuged, and cell pellet may be resuspended in about 100 ml of fresh Dulbecco's Medium containing about 20% fetal bovine serum (Hyclone, Utah, Co). Aliquots of about 100 µl can be added to each well of ten 96-well microtiter plates (Corning, Lowell, MA). About twenty four hours later, about 100 µl DMEM culture medium supplemented with about 1M hypoxanthine (HT), about 4 mM aminopterin and about 160 mM thymidine (HAT) can be added to each microtiter well. Media may be replaced perhaps after about 4 days with complete media (perhaps containing HAT and HT). Over the following about 10 days, media may be removed and replaced with

fresh media with reduced or perhaps even no HAT and HT added. Hybridoma supernatants may be screened by ELISA for antibody reactivity to UPII, and hybridoma clones may then be selected and stabilized perhaps by cloning twice by limiting dilution.

Hybridoma cells referred to as Anti-human UPII hybridoma clone BC21 have been deposited with the American Type Culture Collection (ATCC) under ATCC Patent Deposit Designation No. PTA-13181. Embodiments of the present invention may provide an antibody or fragment thereof produced by the hybridoma deposited at the ATCC and may even include a method for producing a monoclonal antibody by culturing the hybridoma cell which produces the monoclonal antibody capable of specifically recognizing Uroplakin II and even allowing the hybridoma to produce monoclonal antibodies.

ELISA: Host anti-sera immune responses to UPII may be measured by ELISA. For example, a solution of UPII (about 1μg/ml) in phosphate-buffered saline (PBS) may be used to coat about 96-well flat bottom polystyrene plates. The plates may then be blocked with about 1% bovine serum albumin (BSA)–PBS. Either diluted immune sera or hybridoma supernatants may be added and incubated at about 37°C for about 1 hour. After washing the plates with PBS, the plates may be incubated with goat anti-mouse-HRP reagents (Jackson Labs). Incubations may be done at about 37°C for about 30 minutes. ABTS substrate may be added to develop color and the absorbance at about 405 nm (A405) may be measured in a microtiter plate reader.

Isotype of monoclonal antibodies: Anti-UPII antibodies such as the BC21 monoclonal antibody may be isotyped using a mouse monoclonal antibody isotyping kit (Invitrogen, Carlsbad CA). For example, about 100 μl of supernatant from mouse monoclonal antibody [BC21] cells may be added to the plate coated goat anti mouse IgGl, IgG2A, IgG2B, IgG3, IgM, and IgA. After about 30 minutes incubation, the plate may be washed 3 times with PBS and may be incubated with goat anti mouse Ig-HRP reagent. ABTS substrate may be added to develop color and the absorbance at about 405 nm (A405) may be measured in a microtiter plate reader. The BC21 clone may be tested for isotype and may be identified as a mouse IgG1/kappa.

Antibody Production and purification: The selected hybridoma cells from clone BC21 may be cultured with DMEM culture medium supplemented with about 10% FBS or any serum-free medium. The culture supernatants may be further purified by protein A

affinity column. The hybridoma cells may also be injected into pristane-primed BALB/c mice to produce antibody ascites. The antibody ascites may be further purified by protein A affinity column. IgG concentration may be measured spectrophotometrically using the extinction coefficient for human IgG of about 1.4 (about 0.1% at about 280nm). The purity of IgG may be determined by SDS-PAGE.

Cross-reactivity tested by Western Blotting: The purified monoclonal antibody [BC21] may be characterized by Western Blotting. Full-length UPII or UPIII protein may be subjected to protein gel electrophoresis using about 4 to about 12% SDS-PAGE with Tris-glycine buffer and may be transferred onto nitrocellulose filters in Tris-glycine buffer. Proteins on the blots may be visualized by incubating the BC21 antibody for about 60 minutes in room temperature after blocking with blocking buffer, perhaps followed by incubating with peroxidase-conjugated goat anti-mouse immnoglobulins.

Determination of VH and VL sequences: Total RNA may be extracted from hybridomas using Qiagen kit (USA, Gaithersburg, MD) as per the manufacturer's instructions. First-round RT-PCR may be carried out with QIAGEN® OneStep RT-PCR Kit. RT-PCR may be performed with primer sets specific for the heavy and light chains. For each RNA sample, about 12 individual heavy chain and about 11 light chain RT-PCR reactions can be set up using degenerate forward primer mixtures covering the leader sequences of variable regions. Reverse primers may be located in the constant regions of heavy and light chains. Restriction sites may not be engineered into the primers. The RT-PCR products from the first-round reactions may be amplified in the second-round PCR. About 12 individual heavy chain and about 11 light chain RT-PCR reactions can be set up using semi-nested primer sets specific for antibody variable regions. The amplified cDNAs can be gel purified and may then be sequenced.

[BC21] Variable Domains were sequenced to provide isolated polynucleotides that comprise nucleic acid sequences encoding the amino acid sequences of one or more of the CDRs of the light and/or heavy chain variable regions of a monoclonal antibody described herein that binds to the UP II epitope **LSPALTESLLVALPP** identified as SEQ ID NO: 4. The sequence of the variable region of the heavy chain is identified as SEQ ID NO: 1 and the sequence of the variable region of the light chain is identified as SEQ ID NO: 2 or SEQ ID NO: 3. An antibody or fragment thereof may include a polypeptide of the amino acid

sequence encoded by the nucleic acid sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2 and/or SEQ ID NO: 3. An antibody or fragment thereof may include a light chain variable region having an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 2 and/or SEQ ID NO: 3 and may even include a heavy chain variable region having an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1. An antibody or fragment thereof may specifically bind to at least one polypeptide of an amino acid sequence of SEQ ID NO: 4. As mentioned herein, a fragment thereof may include an antigen binding fragment thereof.

In embodiments, an antibody or fragment thereof, or even an isolated and purified nucleic acid sequence may have an amino acid sequence of at least about 70% identical to an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1 and/or SEQ ID NO: 2 and/or SEQ ID NO: 3. An antibody or fragment thereof may specifically binds to at least one polypeptide with an amino acid sequence that is at least about 70% identical to residues of SEQ ID NO: 4. Other percentages may include, but are not limited to, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and perhaps even at least about 99%, or the like.

Epitope Mapping of the mouse anti-UPII [BC21] Binding Sequence: In order to determine the peptide sequence of UP II that is recognized by anti-UPII antibodies such as BC21, epitope mapping may be conducted perhaps using two assays: direct ELISA and even dot blot. In an ELISA assay, the sensitivity and specificity of the anti-UP II [BC21] antibody may be determined by measuring the antibody titer at about 1:500 and about 1:1000. Overlapping peptides at a length of about 15 amino acids each, covering the human UP II protein sequence from perhaps 26 to 155 amino acids, may be used to determine the preferred sequence of BC21 binding.

The epitope for BC21 was shown to be included in the residues 36-50 amino acids of UPII, which is **LSPALTESLLVALPP** identified as SEQ ID NO: 4. The epitope of the

mouse monoclonal UPII antibody, or a portion thereof, may be a useful antigen for the production of new monoclonal antibodies, including production in species other than mouse (e.g. rabbit, goat, horse, chicken, etc.). Of course, a polyclonal antibody may specifically bind to an epitope in SEQ ID NO: 4 which relates to residues 36-50 of the Uroplakin II protein.

For direct ELISA protocol, the plates may be first coated with about 100µl of UP II peptides at about 5µg/mL in coating buffer (pH about 9.5) overnight at about 4°C, followed by blocking (about 3% BSA) at about 200 µl/well for about 1 hour at room temperature. The plates may be incubated with purified UP II antibody at about 100 ng/mL and about 200 ng/mL separately for about 1 hour at about room temperature on an ELISA-plate shaker. Then the plates may be washed perhaps five times with PBST (about 300µl/well) followed by the addition of goat anti-mouse IgG-HRP to the plates and incubation for about 1 hour on a plate-shaker. The plates may then be washed with PBST (about 300µl/well) and blotted to dry, and TMB may be added at about 100µl /well, developed for about 5 min on a shaker, and may even be followed by a stop solution (about 50µl /well). Absorbance may be measured at about 450nm on an ELISA plate reader perhaps according to the manufacturer's recommendation.

For the dot blot assay, a nitrocellulose membrane may be blotted with about 1 µl at a concentration of about 1mg/ml the peptide, quadruplicates per peptide. This membrane may be incubated for about 1 hour at room temperature until it may be completely dry. The membrane may be blocked with about 3% BSA in TBST (e.g., about 50 mM Tris, about 0.5 M NaCl, about 0.05% Tween-20, pH about 7.4) for about 1 hour at room temperature, then mouse anti UP II antibody [BC21] may be added at about 200ng/ml for about 1hr at RT in TBST. Then the membrane may be washed for about 3 times (about 10 minutes each) in TBST on an orbital shaker, followed by incubating with secondary antibody goat anti mouse IgG1-AP for about 1 hour at room temperature in TBST. The membrane may be washed perhaps about 3 times (about 10 minutes each) in TBST on a rocker. The binding may be detected by adding Western Glo Chemiluminescent detection reagents and exposing to film.

IHC method with anti-UP II BC21: Immunohistochemistry using anti-UPII antibodies such as the mouse monoclonal anti-UP II antibody BC21 may be performed on formalin-fixed paraffin embedded (FFPE) tissue samples using procedures generally known

to those in the art, as generally exemplified by the following non-limiting examples (e.g., washes with Tris-buffered saline, pH about 7.6, between steps):

1) Sections (~5μm) of formalin fixed paraffin-embedded tissues may be mounted on commercially available microscope slides perhaps coated with polylysine.
2) Sections may be deparaffinized (using xylenes or a xylene-substitute) and may be rehydrated perhaps through a series of alcohol/water solutions, perhaps followed by blocking of endogenous peroxidases perhaps with about 3% hydrogen peroxide solution.
3) Samples may be subjected to heat-induced antigen retrieval using a citrate buffer in a pressure cooker (Reveal, Decloaking Chamber; Biocare Medical) and may be heated to about 125°C for about 30 seconds. [Other antigen retrieval methods known to those skilled in the art (e.g., steamer, microwave oven, enzyme, or the like) may also be acceptable.] Tissues may be allowed to cool for about 10 minutes and then may be rinsed with deionized water.
4) The UP II antibody BC21 may be applied in a phosphate-buffered solution (pH about 6.0) with bovine serum albumin as carrier protein for about 30 minutes.
5) Detection of the UP II antibody perhaps with a horseradish peroxidase (HRP) conjugated secondary antibody (MACH 4 Universal HRP-Polymer Detection, Biocare Medical) may be accomplished in two steps. An initial application of a rabbit anti-mouse IGg antibody for about 10 minutes may be followed by incubation with a goat anti-rabbit-HRP conjugate for about 10 minutes.
6) In perhaps a final detection step, 3,3'-diaminobenzidine (DAB) in buffer perhaps containing about 0.02% hydrogen peroxide (Betazoid DAB, Biocare Medical) may be applied. The oxidation of DAB through an HRP-mediated mechanism may result in precipitation of a brown, chromogenic product, perhaps allowing identification of sites of UP II expression.
7) Slides may be briefly counterstained perhaps in a modified Mayer's hematoxylin.

Results of IHC Staining with mouse monoclonal anti-UP II antibody BC21: Using the above protocol, a variety of normal and neoplastic tissues were evaluated for UP II expression using BC21 and compared to staining patterns using a mouse monoclonal anti-UP III antibody (BC17, Biocare Medical). Both antibodies were optimized for titer (e.g., concentration) using methods well known to those in the art. For example, various

antibody titers were evaluated to maximize staining intensity, perhaps while minimizing or even eliminating background staining. For each antibody, the titer that provided the maximum staining intensity, perhaps with the minimal background staining, was used.

Figures 1-8 shows several examples of staining of bladder transitional cell carcinoma by anti-UP II antibody (BC21), in comparison to staining with anti-UP III antibody (BC17), on a serial section of the same specimen.

Table 1 shows the sensitivity of anti-UP II antibody (BC21) staining 178 specimens of bladder cancer (e.g., transitional cell carcinoma (TCC) and papillary TCC), using a tissue microarray (TMA). Employing a cut-off of ≥ about 5% of tumor cells staining as the criteria to determine a case as "positive" for UP II, and conversely < about 5% of tumor cells staining as the criteria to determine a case "negative," 137 of 178 (about 77%) were found to be positive for UP II (BC21). Diagnosis of tumors of higher grade can sometimes be a challenge. In these specimens, anti-UP II antibody (BC21) identified 68 of 83 (about 82%) of Grade II tumors, and 25 of 44 (about 57%) of Grade III tumors.

Table 1: Anti-UP II Antibody (BC21) on Bladder cancer (TCC and Papillary TCC) TMA

Grade	Number of Specimens	Number of Positive Specimens	% Positive	Number of Negative Specimens	% Negative
Grades I, II & III	178	137	77%	41	23%
Grade II	83	68	82%	15	18%
Grade III	44	25	57%	19	43%

The greater sensitivity of anti-UP II antibody (BC21), compared to anti-UP III antibody (BC17), was demonstrated by staining the same 59 specimens of TCC of Grades I, II and III with each antibody (Table 2). Using the same criteria, anti-UP II antibody (BC21) identified 46 specimens as positive (about 78%), compared to 33 specimens (about 56%) determined to be positive with anti-UP III antibody (BC17). In Grade II specimens, anti-UP II antibody (BC21) and anti-UP III antibody (BC17) demonstrated sensitivities of about 77% (27 of 35) and about 54% (19 of 35), respectively. In Grade III specimens, anti-UP II antibody (BC21) and anti-UP III antibody (BC17) demonstrated a similar sensitivity of

about 64% (7 of 11). In many comparisons, anti-UP II antibody (BC21) provided a darker staining than anti-UP III antibody (BC17).

Table 2: Comparison of anti-UP II antibody (BC21) and anti-UP III antibody (BC17) on Bladder cancer (TCC and Papillary TCC) TMA

Antibody	Grade	Number of Specimens	Number of Positive Specimens	% Positive	Number of Negative Specimens	% Negative
BC21	Grades I, II & III	59	46	78%	13	22%
BC17	Grades I, II & III	59	33	56%	26	44%
BC21	Grade II	35	27	77%	8	23%
BC17	Grade II	35	19	54%	16	46%
BC21	Grade III	11	7	64%	4	36%
BC17	Grade III	11	7	64%	4	36%

Anti-UP II antibody (BC21) may be highly specific perhaps when evaluated on a variety of normal (Table 3) and even neoplastic (Table 4) tissues. Bladder and ureter may be the only normal tissue to stain positive with UP II (BC21). Such staining may be expected, perhaps considering that the known expression of UP II in normal urothelium anti-UP II antibody (BC21) may not stain any other normal or neoplastic tissues, which may demonstrate its high specificity.

Table 3: Anti-UP II antibody (BC21) staining of normal tissues

Tissue	# cases	Number of positive cases
Adrenal gland	3	0
Bladder	7	5
Bone marrow	1	0
Eye	2	0
Breast	3	0
Cerebellum	3	0
Cerebral cortex	3	0
Fallopian tube	3	0

GI-Esophagus	3	0
GI-Stomach	3	0
GI-Small intestine	3	0
GI-Colon	3	0
GI-Rectum	3	0
Heart	3	0
Kidney	6	0
Liver	3	0
Lung	3	0
Ovary	3	0
Pancreas	3	0
Parathyroid	1	0
Pituitary gland	2	0
Placenta	3	0
Prostate	3	0
Skin	2	0
Spinal cord	2	0
Spleen	2	0
Striated muscle	3	0
Testis	3	0
Thymus	3	0
Thyroid	3	0
Tonsil	3	0
Ureter	3	3
Uterus-cervix	3	0
Uterus-endometrium	3	0

Table 4: Anti-UP II antibody (BC21) staining of various tumor tissues

Tumor Type	Number of cases	Number of Positive Cases
Prostate cancer	10	0
Lung cancer	20	0
Breast cancer	10	0
Colon cancer	30	0
Renal cancer	5	0

Anti-UPII antibodies such as the monoclonal mouse anti-UP II antibody (BC21) may offer distinct advantages with its improved sensitivity, perhaps even as compared to

monoclonal mouse anti-UP III antibody (BC17). Figures 1-8 show examples of comparisons of BC21 with BC17 staining serial sections of the same specimen of bladder TCC, perhaps demonstrating the greater sensitivity of BC21. For example, the specimen of Figures 1 and 2 may exhibit strong membrane and cytoplasmic staining with BC21 (Figure 1), while the staining of BC17 may be minimal in this case (Figure 2). In Figures 3 and 4, a strong and widespread staining of BC21 may be observed (Figure 3); whereas only sparse, focal staining may be observed on the same specimen with BC17 (Figure 4). Similarly, the specimen of Figures 5 and 6 may display strong staining with BC21 (Figure 5), but may have only limited staining with BC17 (Figure 6). Finally, Figures 7 and 8 show a specimen that may also exhibit a strong staining with BC21 (Figure 7); in contrast, BC17 may be negative on this same specimen (Figure 8).

These examples demonstrate cases where a pathologist may have been able to definitively identify the presence of urothelial carcinoma with an anti-UP II antibody such as BC21, which would not have been possible with a less sensitive antibody, such as with BC17 (Figures 1, 2, 7 and 8). Or, the ambiguous results with an anti-UP III antibody, such as BC17 may have led to an equivocal diagnosis that lacks confidence, whereas an anti-UP II antibody, such as BC21, may offer a clear, unambiguous result (Figures 3, 4, 5 and 6).

The minimal staining observed with BC17 in Figures 3, 4, 5 and 6 may provide excellent examples of the challenge that may be faced by pathologists when using a less sensitive antibody; specifically, when the staining observed may be sparse and light, it may be difficult to determine with confidence if this is true positive staining, signaling the presence of UP II and perhaps indicative of urothelial carcinoma, or if it is a misleading staining artifact and should be dismissed. The ambiguity associated with a less sensitive antibody may lead to equivocal, or even incorrect diagnoses and patients with urothelial carcinoma may not receive appropriate treatment in a timely fashion. In contrast, an anti-UP II antibody, such as BC21, may offer a significant advantage for diagnosis with its increased sensitivity. An anti-UP II antibody, such as BC21, may result in strong, clear staining of urothelial carcinoma that may allow a pathologist to definitively return a diagnosis of urothelial carcinoma, perhaps allowing a patient to expeditiously receive the most appropriate treatment.

Results of Western blots with mouse monoclonal anti-UP II antibody BC21

Binding of BC21 to UP II protein may be demonstrated by Western blot (Figure 9A). The absence of similar binding of BC21 to UP III protein may also be shown by Western blot (Figure 9A). Conversely, the anti-UP III antibody BC17 may not bind UP II protein, but may recognize UP III protein (Figure 9B).

In some embodiments of the present invention, anti-UPII antibodies such as the mouse monoclonal anti-UP II antibody BC21 may be suitable for use in many variations of the above protocols and other methods known to those in the art. Specimens stained with BC21 may be archived using a permanent mounting media and a coverslip. The antibody BC21 may also be used in an automated staining instrument, using standard protocols. One can also envision the use of many alternative detection methods (e.g., fluorescence), detection enzymes (e.g., alkaline phosphatase (AP), beta-galactosidase, or the like), and perhaps even chromogens (e.g., 3-amino-9-ethylcarbazole, 5-bromo-4-chloro-3-indolyl phosphate, 3,3′,5,5′-tetramethylbenzidine, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, or the like), generally known to those in the art.

An epitope of an anti-UPII antibodies such as mouse monoclonal anti-UP II antibody, or a portion thereof, may be a useful antigen for the production of new monoclonal antibodies, including production in species other than mouse (e.g. rabbit, goat, horse, chicken, etc.) as one skilled in the art would understand. A monoclonal antibody for Uroplakin III may include but is not limited to a mouse monoclonal antibody, a rabbit monoclonal antibody, a goat monoclonal antibody, a horse monoclonal antibody, a chicken monoclonal antibody, a humanized monoclonal antibody, a chimeric antibody, any combination thereof, or the like. In other embodiments, a polyclonal antibody for Uroplakin III may include but is not limited to rabbit polyclonal antibody, mouse polyclonal antibody, a goat polyclonal antibody, a horse polyclonal antibody, a chicken polyclonal antibody, a humanized polyclonal antibody, any combination thereof, or the like. In yet other embodiments, an antibody may be an isolated antibody.

While the use of anti-UPII antibodies such as BC21 in immunohistochemistry of formalin-fixed paraffin embedded tissues may be described here, its utility in other immunoassays may be readily envisioned and all are included in this application. In particular, it may be well known that many of the same reagents used in IHC of FFPE may

also be used in IHC of frozen-tissue sections. Anti-UPII antibodies such as BC21 may also be useful in other immunoassays, including ELISA, perhaps using generally known methods.

In another aspect of the invention, perhaps related to IHC, an anti-UP II antibody may be used in conjunction with one or more additional primary antibodies as part of a cocktail, to perform a "double-stain" procedure (also described as multi-stain or even multiplex). Such "double-stain" procedures may be generally well known in the art; however, the best combinations of primary antibodies for a particular diagnostic application may not be known.

In this method, anti-UPII antibodies such as a mouse monoclonal anti-UP II antibody BC21 could be combined with one or more antibodies in a primary antibody cocktail. At least one of the additional antibodies could be derived from a species other than mouse such as a rabbit antibody or the like. Antibodies may be derived from at least two different species such as but not limited to a mouse host or a rabbit host or the like. Species may include but are not limited to mouse, rabbit, goat, horse, chicken, human, any combination thereof, or the like. The antibodies may be monoclonal or polyclonal. In this manner, the multiple antibodies in the primary antibody cocktail may be differentiated in the subsequent detection and even visualization steps. For example, following incubation of the tissue sample with the primary antibody cocktail, the usual goat anti-mouse antibody conjugated to HRP may be applied perhaps followed by an appropriate chromogen, such as DAB or the like. Subsequently, a second detection step may be performed, using goat anti-rabbit antibody conjugated to AP perhaps followed by an appropriate chromogen, such as Fast Red or the like. In this manner, two or more targets may be identified on the same tissue sample with the resulting two colors. In this specific example, mouse primary antibodies (including BC21) could result in brown (DAB) staining and rabbit primary antibodies could result in red (Fast Red) staining.

The anti-mouse or anti rabbit antibodies comprising the antibody-enzyme conjugates may be derived from a different host species, including, but not limited to mouse, rabbit, chicken, horse, rat, goat, sheep, or the like. A primary antibody may be from a variety of host species, including, but not limited to mouse, rabbit, chicken, horse, rat, goat, sheep, or the like. In embodiments, an antibody may include an antibody-enzyme conjugate and a

primary antibody could be obtained from two different host species. Chromogens other than DAB and/or Fast Red may be used as well.

Multiple alternatives to a double-staining method are possible, including but not limited to the use of more than two antibodies, the use of species other than mouse and rabbit, other chromogens and detection systems, a different order of detection steps, and perhaps even modifications resulting in three or more colors (which may require a denaturing step).

Embodiments of the present invention may provide a composition having at least two antibodies or fragments thereof, perhaps as a cocktail, where at least one of the two antibodies or fragments thereof specifically binds to at least Uroplakin II. This may provide a method for detecting at least two different proteins in a biological sample perhaps by contacting a biological sample with a composition comprising at least two antibodies or fragments thereof, where at least one of the at least two antibodies or fragments thereof may bind specifically to at least Uroplakin II, to form an antigen-antibody complex and an antigen-antibody complex may be detected. A composition may have at least one first primary antibody and at least one second primary antibody. A biological sample may include but is not limited to blood, urine, bladder tissue, urothelial tissue, transitional cell tissue, normal tissue, neoplastic tissue, kidney tissue, ovarian tissue, thyroid tissue, endometrial tissue, renal tissue, tonsil tissue, pancreas tissue, colon tissue, lymph node tissue, neoplastic pancreatic tissue, stomach tissue, prostate tissue, lung tissue and breast tissue, or the like.

At least one of the antibodies or fragments thereof may specifically bind to at least Uroplakin II and may even have a positive indication cut-off value of greater than 1% of stained cells. As mentioned herein, a positive indication cut-off value may provide a percentage of stained cells needed to indicate a positive staining result. Other cut-off value may include but are not limited to greater than about 1% of stained cells, greater than about 2% of stained cells, greater than about 3% of stained cells, greater than about 4% of stained cells, greater than about 5% of stained cells, greater than about 6% of stained cells, greater than about 7% of stained cells, greater than about 8% of stained cells, greater than about 9% of stained cells, and perhaps even greater than about 10% of stained cells, or more, or the like.

In embodiments, the present invention may provide a composition with at least two antibodies or fragments thereof which may be capable of providing different visualization results such as different color results. As discussed in other embodiments, below, a composition may provide that at least one other of an at least two antibodies or fragments thereof may bind specifically to GATA-3, p63, Uroplakin III, PAX8, NKX3.1, PSA, any combination thereof, or the like. Antibodies, compositions thereof, perhaps with anti-Uroplakin II antibodies may provide a detection system including but not limited to urothelial carcinoma detection composition, renal cell carcinoma detection composition, prostate/prostatic carcinoma detection composition, any combination thereof, or the like.

In some embodiments, a single color stain may be used for a primary antibody cocktail. In one example, if the primary antibody cocktail is comprised of antibodies all derived from the same host species, then a single antibody enzyme conjugate may be used to stain for the presence of all of the antibodies with a single color. The presence or absence of each antibody may be determined based on cellular localization, or perhaps such determination is not necessary and the staining may be interpreted effectively without identifying the presence or absence of each individual antibody.

Certain steps of an IHC procedure may be performed sequentially or simultaneously, perhaps by using a cocktail of reagents, as known to those skilled in the art. For example, antibodies described in a primary antibody cocktail may alternatively be applied in sequential steps of one or more antibodies. Similarly, detection reagents may be applied simultaneously in reagent cocktail or separate reagents in sequential steps.

In some embodiments, a first primary antibody may be applied, followed by a first antibody-enzyme conjugate and first chromogen, and then a denaturing step, before proceeding to application of a second primary antibody, followed by a second antibody-enzyme conjugate and a second chromogen. In this manner, a double-stain of two different colors may be achieved using primary antibodies derived from the same species.

Antibodies that may be useful for diagnosis when combined with an anti-UPII antibody such as a mouse monoclonal anti-UP II antibody BC21 in a primary antibody cocktail for use in multi-stain procedures may include:

Table 5

Antibody Cocktail	Utility
UPII + UPIII	Urothelial marker of enhanced sensitivity
UPII + GATA3	Urothelial marker of enhanced sensitivity
UPII + UPIII + GATA3	Urothelial marker of enhanced sensitivity
UPII + PAX8	Differential marker of bladder and kidney
UPII + PAX8 + PSA	Differential marker of bladder, kidney and prostate
UPII + NKX3.1	Differential marker of bladder and prostate
UPII + PAX8 + NKX3.1	Differential marker of bladder, kidney and prostate
UPII + p63	Urothelial marker of enhanced sensitivity and differential marker of bladder and prostate and differential marker of bladder and non-bladder squamous cell carcinoma
UPII + GATA-3 and/or p63 + PAX8 + PSA and/or NKX3.1	Differential marker of bladder, kidney, and prostate cancer
UPII + p40	Urothelial marker of enhanced sensitivity and differential marker of bladder and prostate and differential marker of bladder and non-bladder squamous cell carcinoma

An anti-UPII antibody such as a mouse monoclonal anti-UP II antibody BC21 may be specific for detection of UP II and may be useful in immunohistochemical procedures for diagnosis of several types of cancers in human tissue samples. In particular, anti-UP II antibody such as BC21 has advantages over anti-UP III antibody BC17, including but not limited to greater sensitivity.

Expression levels of UP II protein may be a prognostic marker of patient outcomes in cases of bladder cancer. Determination of UP II expression, using an antibody such as BC21, may aid in identifying patients more likely to experience a positive outcome (e.g. longer survival time, longer time to disease progression, reduced tumor size, or the like), a

positive or good prognosis, or those patients more likely to experience a negative outcome (e.g. shorter survival time, shorter time to disease progression, or the like), a negative or poor prognosis. Determination of UP II expression, using an antibody such as BC21, may also aid in predicting patient response to a particular therapeutic treatment. For example, the level of UPII expression may aid in determining the likelihood that a patient could benefit from a particular pharmaceutical agent, including antibody based therapeutics. Conversely, UP II expression may aid in determining the likelihood that a patient may not benefit from a particular therapeutic treatment.

Alternative embodiments of antibodies that may be useful for diagnosis when combined with an UPII antibody such as a mouse monoclonal UPII antibody BC21 in a primary antibody cocktail for use in multi-stain procedures can include:

Table 6

Antibody Combination (Host Species, cellular localization, stain color*)	Possible Diagnostic Utility	Detection System used in example and Figure No.
UPIII (Mouse, Membrane & cytoplasmic, brown) UPII (Mouse, Membrane & cytoplasmic, brown)	UPII and/or UPIII staining may be observed in urothelial carcinoma.	Goat anti-mouse HRP Figure 10
UPII (Mouse, Membrane & cytoplasmic, red) GATA3 (Rabbit, nuclear, brown)	UPII and/or GATA3 staining may be observed in urothelial carcinoma.	DS#1 Figures 11, 12
UPII (Mouse, Membrane & cytoplasmic, red) UPIII (Mouse, Membrane & cytoplasmic, red) GATA3 (Rabbit, nuclear, brown)	UPII and/or UPIII and/or GATA3 staining may be observed in urothelial carcinoma.	DS#1 Figure 13

Antibody Combination (Host Species, cellular localization, stain color*)	Possible Diagnostic Utility	Detection System used in example and Figure No.
UPII (Mouse, Membrane & cytoplasmic, red) PAX8 (Rabbit, nuclear, brown)	UPII staining may be observed in urothelial carcinoma. PAX8 staining may be observed in renal cell carcinoma.	DS#1 Figures 14, 15, 16, 17
UPII (Mouse, Membrane & cytoplasmic, red) PAX8 (Rabbit, nuclear, brown) PSA (Rabbit, cytoplasmic, brown)	UPII staining may be observed in urothelial carcinoma. PAX8 staining may be observed in renal cell carcinoma. PSA staining may be observed in prostatic carcinoma.	DS#1 Figures 18, 19, 20
UPII (Mouse, Membrane & cytoplasmic, brown) PAX8 (Rabbit, nuclear, red) PSA (Rabbit, cytoplasmic, red)	UPII staining may be observed in urothelial carcinoma. PAX8 staining may be observed in renal cell carcinoma. PSA staining may be observed in prostatic carcinoma.	DS#2 Figures 21, 22, 23
UPII (Mouse, Membrane & cytoplasmic, brown) NKX3.1 (Rabbit, nuclear, red)	UPII staining may be observed in urothelial carcinoma. NKX3.1 staining may be observed in prostatic carcinoma.	DS#2 Figures 24, 25
UPII (Mouse, Membrane & cytoplasmic, brown) PAX8 (Mouse, nuclear, brown) NKX3.1 (Rabbit, nuclear, red)	UPII staining may be observed in urothelial carcinoma. PAX8 staining may be observed in renal cell carcinoma. NKX3.1 staining may be observed in prostatic carcinoma.	DS#2 Figures 26, 27, 28

Antibody Combination (Host Species, cellular localization, stain color*)	Possible Diagnostic Utility	Detection System used in example and Figure No.
UPII (Mouse, Membrane & cytoplasmic, red) p63 (Mouse, nuclear, brown)	UPII staining may be observed in urothelial carcinoma. p63 staining may be observed in urothelial carcinoma.	Goat anti-mouse HRP and Goat anti-mouse AP Figure 29
UPII (Mouse, Membrane & cytoplasmic, brown) p63 (Mouse, nuclear, brown)	UPII staining may be observed in urothelial carcinoma. p63 staining may be observed in normal prostate or PIN.	Goat anti-mouse HRP Figures 30, 31, 32
UPII (Mouse, Membrane & cytoplasmic, red) p40 (Mouse, nuclear, brown)	UPII staining may be observed in urothelial carcinoma. p40 staining may be observed in urothelial carcinoma.	Goat anti-mouse HRP and Goat anti-mouse AP Figure 33

* The listed color of each stain may be a result of a detection system that may include an anti-mouse antibody perhaps conjugated to HRP and even an anti-mouse antibody perhaps conjugated to AP, perhaps even with DAB and Fast Red as chromogens, which may result in brown staining for mouse antibodies and red staining for rabbit antibodies (referred to as DS#2). Alternatively, the detection system may include an anti-mouse antibody perhaps conjugated to AP and even an anti-rabbit antibody perhaps conjugated to HRP, perhaps even with DAB and Fast Red as chromogens, which may result in red staining for mouse antibodies and brown staining for rabbit antibodies (referred to as DS#1). In some instances, two colors may not be necessary because the antigens may be distinguished by cellular localization of staining, or perhaps it is not diagnostically significant to determine which antigen is staining. Other color combinations may be obtained using other detection systems or chromogens and all are meant to be included in this disclosure.

The non-limiting examples of various cocktails listed in Table 6 are examples only and is not intended to suggest that every case of a particular cancer could produce the same result. For example, not all cases of urothelial carcinoma could be positive for UPII and/or

GATA3. Additionally, not all cases of renal cell carcinoma could be positive for PAX8, and the like. Each marker may be reduced or may even be absent in other cases.

In some embodiments, combining UPII with another antibody that stains urothelial tissue, such as UPIII, may be useful, perhaps increasing sensitivity compared to staining with each of the antibodies individually. Figure 10 shows an example of a cocktail of UPIII + UPII staining a specimen of urothelial carcinoma. In some cases, UPII staining may be observed, when perhaps UPIII staining is reduced or absent. In other cases, UPIII staining may be observed, when perhaps UPII staining is reduced or absent.

A cocktail of UPII + GATA3 may also provide increased sensitivity for urothelial carcinoma. A specimen of urothelial carcinoma stained with a cocktail of UPII + GATA3 is shown in Figure 11. In some cases, UPII staining may be observed, when perhaps GATA3 staining is reduced or absent. In other cases, GATA3 staining may be observed, when perhaps UPII staining is reduced or absent.

Combining multiple markers of urothelial carcinoma may further enhance sensitivity. A specimen stained with a cocktail of UPII + GATA3 is shown in Figure 12. The same specimen stained with a cocktail of UPII + UPIII + GATA3 is shown in Figure 13. Perhaps more staining is observed with the UPII + UPIII + GATA3 cocktail, which may result in improved sensitivity.

Combining UPII + PAX8 may be useful for differentiating urothelial carcinoma and renal cell carcinoma. UPII may stain urothelial carcinoma, which is not stained by PAX8 (Figures 14 and 15). In contrast, renal cell carcinoma may be stained by PAX8, but perhaps not by UPII (Figures 16 and 17).

Combining UPII + PAX8 + PSA may be useful for differentiating urothelial carcinoma renal cell carcinoma, and prostate carcinoma. UPII may stain urothelial carcinoma, which is not stained by PAX8 or PSA (Figures 18 and 21). In contrast, renal cell carcinoma may be stained by PAX8, but perhaps not by UPII or PSA (Figures 19 and 22). Additionally, prostate carcinoma may be stained by PSA, but perhaps not by UPII or PAX8 (Figures 20 and 23).

Combining UPII + NKX3.1 may be useful for differentiating urothelial carcinoma and prostate carcinoma. UPII may stain urothelial carcinoma, which is not stained by

NKX3.1 (Figure 24). In contrast, prostate carcinoma may be stained by NKX3.1, but perhaps not by UPII (Figure 25).

Combining UPII + PAX8 + NKX3.1 may be useful for differentiating urothelial carcinoma renal cell carcinoma, and prostate carcinoma. UPII may stain urothelial carcinoma, which is not stained by PAX8 or NKX3.1 (Figure 26). In contrast, renal cell carcinoma may be stained by PAX8, but perhaps not by UPII or NKX3.1 (Figure 27). Additionally, prostate carcinoma may be stained by NKX3.1, but perhaps not by UPII or PAX8 (Figure 28).

A cocktail of UPII + p63 may also provide increased sensitivity for urothelial carcinoma. A specimen of urothelial carcinoma stained with a cocktail of UPII + p63 is shown in Figures 29 and 30. Normal prostate, or perhaps prostatic intraepithelial neoplasia (PIN) may be stained by p63, but perhaps not by UPII (Figures 31 and 32).

A cocktail of UPII + p40 may also provide increased sensitivity for urothelial carcinoma. A specimen of urothelial carcinoma stained with a cocktail of UPII + p40 is shown in Figure 33.

Figure 34 shows a schematic summary of various embodiments of the present invention including a kit (5) which may provide an antibody, fragment thereof, portion thereof, in a composition or even in a cocktail, perhaps even provided from a hybridoma, the antibody (1) or the like may be contacted with a biological sample (2) to form at least one antibody-antigen complex (3) which may then be detected with a detector (4).

As but one example of an immunoassay method, embodiments of the present invention may provide obtaining tissue from an animal or human to be tested (6), fixing or freezing said tissue (7), treating said fixed or frozen tissue to unmask epitopes to Uroplakin III (8), contacting said treated tissue with an antibody or fragment thereof as discussed herein in an amount and under conditions such that an antibody or fragment thereof binds to a Uroplakin III protein if the protein is present in said tissue (9); and perhaps even detecting the presences of said bound antibodies (10), as schematically represented in Figure 35.

The present invention may provide, in embodiments, a diagnostic or even prognostic test kit which may include an antibody or fragment thereof (as discussed herein) with an antibody detection element of the antibody or fragment thereof perhaps when bound to an antigen. This may provide a method of contacting a biological sample with an antibody or

fragment thereof and even detecting binding of, or even the presence of the antibody or fragment thereof bound to a protein or with an antigen in the biological sample perhaps using an antibody detection element. Embodiments may provide an immunoassay method for detecting Uroplakin II protein in a mammal or human perhaps by obtaining a tissue from an animal or a human to be tested, contacting the tissue with an antibody or fragment thereof in accordance with the various embodiments presented herein perhaps in an amount and under conditions such that the antibody or fragment thereof may bind to a Uroplakin II protein if the protein is present in the tissue; and even detecting the presence of bound antibodies. A biological sample may include but is not limited to blood, urine, urothelial tissue, transitional cell tissue, bladder tissue, normal tissue, neoplastic tissue, kidney tissue, ovarian tissue, thyroid tissue, endometrial tissue, renal tissue, tonsil tissue, pancreas tissue, colon tissue, lymph node tissue, neoplastic pancreatic tissue, stomach tissue, prostate tissue, lung tissue, breast tissue, or the like perhaps depending on the antibody or even cocktail being used.

It is noted that use of terms such as UPII, UPII antibody, UPIII, UPIII antibody, BC21, BC17, or the like may related to anti-UPII antibodies, anti-UPIII antibodies, or the like as appropriate as one skilled in the art would understand.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both antibody techniques as well as devices to accomplish the appropriate antibody. In this application, the antibody techniques are disclosed as part of the results shown to be achieved by the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or

equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in device-oriented terminology, each element of the device implicitly performs a function. Apparatus claims may not only be included for the device described, but also method or process claims may be included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims that will be included in any subsequent patent application.

It should also be understood that a variety of changes may be made without departing from the essence of the invention. Such changes are also implicitly included in the description. They still fall within the scope of this invention. A broad disclosure encompassing the explicit embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for any subsequent patent application. It should be understood that such language changes and broader or more detailed claiming may be accomplished at a later date (such as by any required deadline) or in the event the applicant subsequently seeks a patent filing based on this filing. With this understanding, the reader should be aware that this disclosure is to be understood to support any subsequently filed patent application that may seek examination of as broad a base of claims as deemed within the applicant's right and may be designed to yield a patent covering numerous aspects of the invention both independently and as an overall system.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. Additionally, when used or implied, an element is to be understood as encompassing individual as well as plural structures that may or may not be physically connected. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all

actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "detection" or "detector" should be understood to encompass disclosure of the act of "detecting" -- whether explicitly discussed or not -- and, conversely, were there effectively disclosure of the act of "detecting", such a disclosure should be understood to encompass disclosure of a "detector" and even a "means for detecting." Such changes and alternative terms are to be understood to be explicitly included in the description. Further, each such means (whether explicitly so described or not) should be understood as encompassing all elements that can perform the given function, and all descriptions of elements that perform a described function should be understood as a non-limiting example of means for performing that function.

Any law, statutes, regulations, or rules mentioned in this application for patent; or patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. Any priority case(s) claimed by this application is hereby appended and hereby incorporated by reference. In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with a broadly supporting interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary, second edition are hereby incorporated by reference. Finally, all references listed below or in any list of References or other information statement filed with the application are hereby appended and hereby incorporated by reference, however, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

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United States Provisional Application Number 61/706,312 filed September 27, 2012; entitled Systems and Methods for Anti-Uroplakin II Antibodies
Budapest restricted certificate of deposit Budapest treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure international form Receipt in the case of an original deposit issued pursuant to rule 7.3 and viability statement issued pursuant to rule 10.2, Anti-human UPII mouse hybridoma: BC21, PTA-13181, 25 vials, Deposited on Behalf of: Biocare Medical, Inc., Date of Receipt of seeds/strain(s) by the ATCC®: September 6, 2012

Thus, the applicant(s) should be understood to have support to claim and make a statement of invention to at least: i) each of the antibody devices as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative

designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) each system, method, and element shown or described as now applied to any specific field or devices mentioned, x) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, xi) an apparatus for performing the methods described herein comprising means for performing the steps, xii) the various combinations and permutations of each of the elements disclosed, xiii) each potentially dependent claim or concept as a dependency on each and every one of the independent claims or concepts presented, and xiv) all inventions described herein.

With regard to claims whether now or later presented for examination, it should be understood that for practical reasons and so as to avoid great expansion of the examination burden, the applicant may at any time present only initial claims or perhaps only initial claims with only initial dependencies. The office and any third persons interested in potential scope of this or subsequent applications should understand that broader claims may be presented at a later date in this case, in a case claiming the benefit of this case, or in any continuation in spite of any preliminary amendments, other amendments, claim language, or arguments presented, thus throughout the pendency of any case there is no intention to disclaim or surrender any potential subject matter. It should be understood that if or when broader claims are presented, such may require that any relevant prior art that may have been considered at any prior time may need to be re-visited since it is possible that to the extent any amendments, claim language, or arguments presented in this or any subsequent application are considered as made to avoid such prior art, such reasons may be eliminated by later presented claims or the like. Both the examiner and any person otherwise interested in existing or later potential coverage, or considering if there has at any time been any possibility of an indication of disclaimer or surrender of potential coverage, should be aware that no such surrender or disclaimer is ever intended or ever exists in this or any subsequent application. Limitations such as arose in *Hakim v. Cannon Avent Group, PLC*, 479 F.3d 1313 (Fed. Cir 2007), or the like are expressly not intended in this or any subsequent related matter. In addition, support should be understood to exist to the degree required under new

matter laws -- including but not limited to European Patent Convention Article 123(2) and United States Patent Law 35 USC 132 or other such laws-- to permit the addition of any of the various dependencies or other elements presented under one independent claim or concept as dependencies or elements under any other independent claim or concept. In drafting any claims at any time whether in this application or in any subsequent application, it should also be understood that the applicant has intended to capture as full and broad a scope of coverage as legally available. To the extent that insubstantial substitutes are made, to the extent that the applicant did not in fact draft any claim so as to literally encompass any particular embodiment, and to the extent otherwise applicable, the applicant should not be understood to have in any way intended to or actually relinquished such coverage as the applicant simply may not have been able to anticipate all eventualities; one skilled in the art, should not be reasonably expected to have drafted a claim that would have literally encompassed such alternative embodiments.

Further, if or when used, the use of the transitional phrase "comprising" is used to maintain the "open-end" claims herein, according to traditional claim interpretation. Thus, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible. The use of the phrase, "or any other claim" is used to provide support for any claim to be dependent on any other claim, such as another dependent claim, another independent claim, a previously listed claim, a subsequently listed claim, and the like. As one clarifying example, if a claim were dependent "on claim 20 or any other claim" or the like, it could be re-drafted as dependent on claim 1, claim 15, or even claim 25 (if such were to exist) if desired and still fall with the disclosure. It should be understood that this phrase also provides support for any combination of elements in the claims and even incorporates any desired proper antecedent basis for certain claim combinations such as with combinations of method, apparatus, process, and the like claims.

Finally, any claims set forth at any time are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all

of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the description into the claims or vice-versa as necessary to define the matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

CLAIMS

What is claimed is:

1. An antibody or fragment thereof produced by the hybridoma deposited with the American Type Culture Collection (ATCC) under ATCC Patent Deposit Designation No. PTA-13181.

2. A hybridoma cell that is deposited at the American Type Culture Collection (ATCC) under ATCC Patent Deposit Designation No. PTA-13181.

3. A hybridoma cell according to claim 2 and further comprising an antibody or fragment thereof produced by said hybridoma cell.

4. A method for producing a monoclonal antibody according to claim 1 comprising the steps of:

culturing said hybridoma which produces a monoclonal antibody capable of specifically recognizing Uroplakin II; and

allowing said hybridoma to produce the monoclonal antibody.

5. An antibody or fragment thereof comprising a polypeptide of the amino acid sequence encoded by the nucleic acid sequence selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

6. An anti-Uroplakin II antibody or fragment thereof, wherein said antibody or said fragment thereof comprises a light chain variable region comprising the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3 and a heavy chain variable region comprising the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 1.

7. An antibody comprising an amino acid sequence at least about 70% identical to an amino acid sequence encoded by a nucleic acid sequence selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

8. An isolated and purified nucleic acid sequence comprising a nucleic acid sequence that is at least about 70% identical to a sequence selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

9. A sequence according to claim 7 or 8 wherein said at least about 70% identical comprises at least about 70% identical to SEQ ID NO: 1 and at least 70% identical to SEQ ID NO: 2 or SEQ ID NO: 3.

10. A sequence according to claim 9 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

11. An antibody or fragment thereof that specifically binds to at least one polypeptide with an amino acid sequence comprising residues of SEQ ID NO: 4.

12. A composition comprising at least two antibodies or fragments thereof, wherein at least one of said at least two antibodies or fragments thereof binds specifically to at least Uroplakin II.

13. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof which binds specifically to at least Uroplakin II comprises at least one of said at least two antibodies or fragments thereof which binds specifically to Uroplakin II and has a positive indication cut-off value of greater than 1% of stained cells.

14. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof which binds specifically to Uroplakin II comprises at least one of said at least two antibodies or fragments thereof which binds specifically to Uroplakin II and has a positive indication cut-off value selected from a group consisting of:

- greater than about 2% of stained cells;
- greater than about 3% of stained cells;
- greater than about 4% of stained cells;
- greater than about 5% of stained cells;
- greater than about 6% of stained cells;
- greater than about 7% of stained cells;
- greater than about 8% of stained cells;

- greater than about 9% of stained cells; and
- greater than about 10% of stained cells.

15. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof comprises a light chain variable region comprising the amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3 and a heavy chain variable region comprising the amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1.

16. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof comprises a light chain variable region comprising an amino acid sequence that is identical to at least about 70% of an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3 and a heavy chain variable region comprising the amino acid sequence that is at least about 70% of an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1.

17. A composition according to claim 16 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

18. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof specifically binds to at least one polypeptide with an amino acid sequence comprising residues of SEQ ID NO: 4.

19. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof specifically binds to at least one polypeptide with an amino acid sequence that is at least about 70% identical to residues of SEQ ID NO: 4.

20. A composition according to claim 19 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

21. A composition according to claim 12 wherein said at least one of said at least two antibodies or fragments thereof comprises an antibody or fragment thereof produced by the hybridoma deposited with the American Type Culture Collection (ATCC) under ATCC Patent Deposit Designation No. PTA-13181.

22. A composition according to claim 12 wherein said composition comprises a primary antibody cocktail.

23. A composition according to claim 12 wherein said at least two antibodies or fragments thereof are derived from at least two different species.

24. A composition according to claim 23 wherein said at least two different species is selected from a group consisting of mouse, rabbit, goat, horse, chicken, human, and any combination thereof.

25. A composition according to claim 12 wherein said at least two antibodies or fragments thereof comprises a double-stain procedure.

26. A composition according to claim 12 wherein said at least two antibodies or fragments thereof are capable of providing different visualization results.

27. A composition according to claim 26 wherein said visualization results comprises color results.

28. A composition according to claim 12 wherein said at least one other of said at least two antibodies or fragments thereof binds specifically to an antigen selected from a group consisting of GATA-3, p63, Uroplakin III, PAX8, NKX3.1, PSA, p40, and any combination thereof.

29. A composition according to claim 12 wherein at least two antibodies or fragments thereof each bind specifically to proteins selected from a group consisting of:
- Uroplakin II and GATA-3;
- Uroplakin II and p63;
- Uroplakin II and Uroplakin III;
- Uroplakin II and PAX8;
- Uroplakin II and NKX3.1;
- Uroplakin II and PSA;
- Uroplakin II and Uroplakin III and GATA-3;
- Uroplakin II and PAX8 and PSA;
- Uroplakin II and PAX8 and NKX3.1; and
- Uroplakin II and p40.

30. A composition according to claim 12 wherein said composition comprises a detection composition selected from a group consisting of urothelial carcinoma detection composition, renal cell carcinoma detection composition, prostate carcinoma detection composition, and any combination thereof.

31. A monoclonal antibody or fragment thereof which binds specifically to Uroplakin II and has a positive indication cut-off value of greater than 1% of stained cells.

32. A monoclonal antibody or fragment thereof according to claim 31 wherein said positive indication cut-off value is selected from a group consisting of:
- greater than about 2% of stained cells;
- greater than about 3% of stained cells;
- greater than about 4% of stained cells;
- greater than about 5% of stained cells;
- greater than about 6% of stained cells;
- greater than about 7% of stained cells;
- greater than about 8% of stained cells;
- greater than about 9% of stained cells; and
- greater than about 10% of stained cells.

33. An antibody according to claim 5, 6, 11, 12, or 31 wherein said antibody or fragment thereof is produced by the hybridoma cells that are deposited at the American Type Culture Collection under ATCC Patent Deposit Designation No. PTA-13181.

34. An antibody according to claim 1, 3, 11, or 31 wherein said antibody or fragment thereof comprises a polypeptide of the amino acid sequence encoded by the nucleic acid sequence selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

35. An antibody according to claim 1, 3, 11, or 31 wherein said antibody or fragment thereof comprises a polypeptide of the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2 or SEQ ID NO: 3.

36. An antibody according to claim 1, 3, 11, or 31 wherein said antibody or fragment thereof comprises a polypeptide that is at least about 70% identical to an amino acid sequence encoded by the nucleic acid sequence selected from a group consisting of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3.

37. An antibody according to claim 1, 3, 11, or 31 wherein said antibody or fragment thereof comprises a polypeptide that is at least about 70% identical to an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 1 and SEQ ID NO: 2 or SEQ ID NO: 3.

38. An antibody according to claim 1, 3, 5, or 31 wherein said antibody or fragment thereof specifically binds to at least one polypeptide with an amino acid sequence comprising residues of SEQ ID NO: 4.

39. An antibody according to claim 1, 3, 5, or 31 wherein said antibody or fragment thereof specifically binds to at least one polypeptide with an amino acid sequence that is at least about 70% identical to residues of SEQ ID NO: 4.

40. An antibody according to claim 39 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least

about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

41. An antibody according to claim 1, 3, 5, 6, 11, 12, or 31 wherein said antibody comprises a monoclonal antibody.

42. An antibody according to claim 41 wherein said monoclonal antibody has a positive indication cut-off value of greater than 1% of stained cells.

43. An antibody according to claim 42 wherein said positive indication cut-off value is selected from a group consisting of:

- greater than about 2% of stained cells;
- greater than about 3% of stained cells;
- greater than about 4% of stained cells;
- greater than about 5% of stained cells;
- greater than about 6% of stained cells;
- greater than about 7% of stained cells;
- greater than about 8% of stained cells;
- greater than about 9% of stained cells; and
- greater than about 10% of stained cells.

44. An antibody according to claim 41 wherein said monoclonal antibody is selected from a group consisting of a mouse monoclonal antibody, a rabbit monoclonal antibody, a goat monoclonal antibody, a horse monoclonal antibody, a chicken monoclonal antibody, a humanized monoclonal antibody, a chimeric antibody, and any combination thereof.

45. An antibody according to claim 1, 3, 5, 6, 11, 12, or 31 wherein said antibody comprises a polyclonal antibody.

46. An antibody according to claim 45 wherein said polyclonal antibody is selected from a group consisting of rabbit polyclonal antibody, mouse polyclonal antibody, a goat polyclonal antibody, a horse polyclonal antibody, a chicken polyclonal antibody, a humanized polyclonal antibody, and any combination thereof

47. An antibody according to claim 1, 3, 5, 6, 11, 12, or 31 wherein said antibody comprises an isolated antibody.

48. An antibody according to claim 1, 3, 5, 6, 11, 12, or 31 wherein said fragment thereof comprises an antigen binding fragment thereof.

49. An antibody according to claim 1, 3, 5, 6, 11, 12, or 31 and further comprising a label attached to said antibody or fragment thereof.

50. A cancer diagnostic agent which comprises said antibody or fragment thereof according to claim 1, 3, 5, 6, 11, 12, or 31 conjugated with a label.

51. An antibody according to claim 49 wherein said label is selected from a group consisting of a radioactive element, magnetic particles, radioisotope, fluorescent dye, enzyme, toxin, signal, stain, detection enzymes, horseradish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase, chromogens, Fast Red, 3,3'-diaminobenzidine, 3-amino-9-ethylcarbazole, 5-bromo-4-chloro-3-indolyl phosphate, 3,3′,5,5′-tetramethylbenzidine, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, and any combination thereof.

52. An antibody according to claim 50 wherein said label is selected from a group consisting of a radioactive element, magnetic particles, radioisotope, fluorescent dye, enzyme, toxin, signal, stain, detection enzymes, horseradish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase, chromogens, Fast Red, 3,3'-diaminobenzidine, 3-amino-9-ethylcarbazole, 5-bromo-4-chloro-3-indolyl phosphate, 3,3′,5,5′-tetramethylbenzidine, 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, and any combination thereof.

53. A diagnostic or prognostic test kit comprising:

- an antibody or fragment thereof according to claim 1, 3, 5, 6, 11, 12, or 31; and
- an antibody detection element of said antibody or said fragment thereof when bound to an antigen.

54. A method for detecting Uroplakin II in a biological sample using the kit of claim 53 comprising the steps of:

- contacting a biological sample with said antibody or fragment thereof; and
- detecting binding of said antibody or said fragment thereof with an antigen in said biological sample using said antibody detection element.

55. An antibody according to claim 1, 3, 5, 6, or 11 wherein said antibody or said fragment thereof specifically binds to Uroplakin II.

56. Use of the antibody or fragment thereof or composition according to claim 1, 3, 5, 6, 11, or 31 to detect cancer.

57. Use of the antibody or fragment thereof or composition according to claim 1, 3, 5, 6, 11, or 31 to diagnose or prognose cancer.

58. Use of the antibody or fragment thereof or composition according to claim 1, 3, 5, 6, 11, or 31 to predict outcome of treatment of cancer.

59. Use of the antibody or fragment thereof or composition according to claim 1, 3, 5, 6, 11, or 31 to assess efficacy of treatment of cancer.

60. Use of the antibody or fragment thereof or composition according to claim 1, 3, 5, 6, 11, or 31 to predict recurrence of cancer.

61. The antibody or fragment thereof or composition according to claim 56 wherein said use of said antibody or fragment thereof or composition is performed on an automated staining device.

62. The antibody or fragment thereof or composition according to claim 56 wherein said detecting is made manually.

63. The antibody or fragment thereof or composition according to claim 56 wherein said detecting is made automatically.

64. The antibody or fragment thereof or composition according to claim 56 wherein said detecting is made by image analysis.

65. A method for detecting a protein to which an antibody or fragment thereof of claim 1, 3, 5, 6, 11, 12, or 31 binds to a biological sample, comprising the steps of contacting a biological sample with the antibody or fragment thereof; and detecting the presence of the antibody or fragment thereof bound to the protein in the biological sample.

66. A method according to claim 65 wherein said biological sample comprises blood, urine, urothelial tissue, transitional cell tissue, and bladder tissue.

67. A method according to claim 65 wherein said biological sample is selected from a group consisting of a normal tissue, neoplastic tissue, bladder tissue, kidney tissue, ovarian tissue, thyroid tissue, endometrial tissue, renal tissue, tonsil tissue, pancreas

tissue, colon tissue, lymph node tissue, stomach tissue, prostate tissue, lung tissue and breast tissue.

68. A method according to claim 65 wherein said detecting said presence of the antibody or fragment thereof bound to the protein is performed on an automated staining device.

69. A method according to claim 65 wherein said detecting said presence of the antibody or fragment thereof bound to the protein is made manually.

70. A method according to claim 65 wherein said detecting said presence of the antibody or fragment thereof bound to the protein is made automatically.

71. A method according to claim 65 wherein said detecting said presence of the antibody or fragment thereof bound to the protein is made by image analysis.

72. A method according to claim 65 wherein said detecting comprises a method selected from a group consisting of immunohistochemistry (IHC), IHC of FFPE, IHC of frozen-tissue sections, immunocytochemistry, and ELISA.

73. A method for detecting at least two different proteins in a biological sample, comprising the steps of:

- contacting said biological sample with a composition comprising at least two antibodies or fragments thereof, wherein at least one of said at least two antibodies or fragments thereof binds specifically to at least Uroplakin II, to form an antigen-antibody complex; and
- detecting said antigen-antibody complex.

74. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof binds specifically to at least Uroplakin II comprises at least one of said at least two antibodies or fragments thereof binds specifically to at least Uroplakin II and has a positive indication cut-off value of greater than 1% of stained cells.

75. A method according to claim 74 wherein said positive indication cut-off value is selected from a group consisting of:

- greater than about 2% of stained cells;
- greater than about 3% of stained cells;
- greater than about 4% of stained cells;

- greater than about 5% of stained cells;
- greater than about 6% of stained cells;
- greater than about 7% of stained cells;
- greater than about 8% of stained cells;
- greater than about 9% of stained cells; and
- greater than about 10% of stained cells.

76. A method according to claim 73 wherein said at least two antibodies or fragments thereof comprise at least one first primary antibody and at least one second primary antibody.

77. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof comprises a light chain variable region comprising the amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3 and a heavy chain variable region comprising the amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1.

78. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof comprises a light chain variable region comprising an amino acid sequence that is identical to at least about 70% of an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3 and a heavy chain variable region comprising the amino acid sequence that is at least about 70% of an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 1.

79. A method according to claim 78 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

80. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof specifically binds to at least one polypeptide with an amino acid sequence comprising residues of SEQ ID NO: 4.

81. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof specifically binds to at least one polypeptide with an amino acid sequence that is at least about 70% identical to residues of SEQ ID NO: 4.

82. A method according to claim 81 wherein said at least about 70% identical comprises a percentage selected from a group consisting of at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%.

83. A method according to claim 73 wherein said at least one of said at least two antibodies or fragments thereof comprises an antibody or fragment thereof produced by the hybridoma deposited with the American Type Culture Collection (ATCC) under ATCC Patent Deposit Designation No. PTA-13181.

84. A method according to claim 73 wherein said composition comprises a primary antibody cocktail.

85. A method according to claim 73 wherein said at least two antibodies or fragments thereof are derived from at least two different species.

86. A method according to claim 85 wherein said at least two different species is selected from a group consisting of mouse, rabbit, goat, horse, chicken, human, and any combination thereof.

87. A method according to claim 73 and further comprising the step of double-staining said biological sample.

88. A method according to claim 73 and further comprising the step of providing different visualization results.

89. A method according to claim 88 wherein said visualization results comprises color results.

90. A method according to claim 73 wherein at least two antibodies or fragments thereof each bind specifically to proteins selected from a group consisting of:

- Uroplakin II and GATA-3;
- Uroplakin II and p63;
- Uroplakin II and Uroplakin III;
- Uroplakin II and PAX8;
- Uroplakin II and NKX3.1;
- Uroplakin II and PSA;
- Uroplakin II and Uroplakin III and GATA-3;
- Uroplakin II and PAX8 and PSA;
- Uroplakin II and PAX8 and NKX3.1; and
- Uroplakin II and p40.

91. A method according to claim 73 wherein said step of detecting said antigen-antibody complex comprises the step of detecting a formation of at least two antigen-antibody complexes on said sample, wherein said first antibody specifically binds to Uroplakin II, wherein said second antibody specifically binds to an antigen selected from a group consisting of: GATA-3, p63, Uroplakin III, PAX8, NKX3.1, PSA, p40, and any combination thereof.

92. An immunoassay method for detecting Uroplakin II protein in an animal or human comprising the steps of:

- obtaining tissue from an animal or human to be tested;
- contacting said tissue with an antibody or fragment thereof according to claim 1, 3, 5, 6, 11, 12, or 31, in an amount and under conditions such that said antibody or fragment thereof binds to a Uroplakin II protein if said protein is present in said tissue; and
- detecting the presence of said bound antibodies.

93 An immunoassay method for detecting Uroplakin II protein in an animal or human according to claim 92 and further comprising the step of fixing or freezing said tissue.

94. An immunoassay method for detecting Uroplakin II protein in an animal or human according to claim 93 and further comprising the step of treating said fixed or frozen tissue to unmask epitopes to Uroplakin II.

95. An immunoassay method for detecting Uroplakin II protein according to claim 92 and further comprising detecting said Uroplakin II protein in said animal or human with a method selected from a group consisting of immunohistochemistry (IHC), IHC of FFPE, IHC of frozen-tissue sections, immunocytochemistry, and ELISA.

96. An isolated preparations of antibodies which specifically bind to an Uroplakin II protein wherein said antibodies bind to an epitope in peptide of SEQ NO: 4 (residues 36-50 of said Uroplakin II protein).

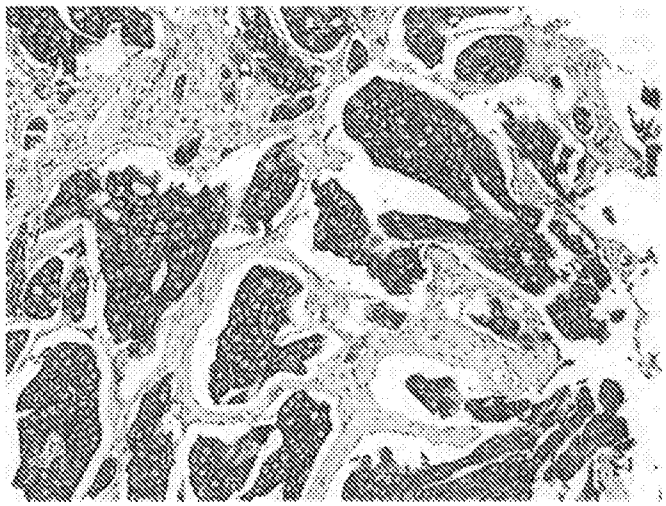

FIG. 1

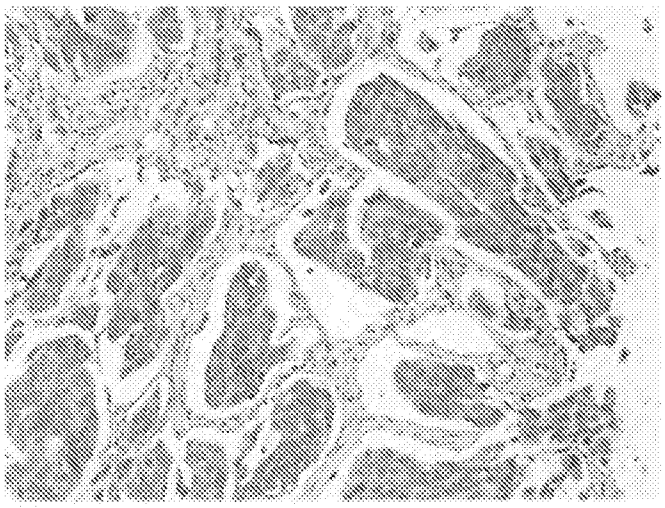

FIG. 2

FIG. 3

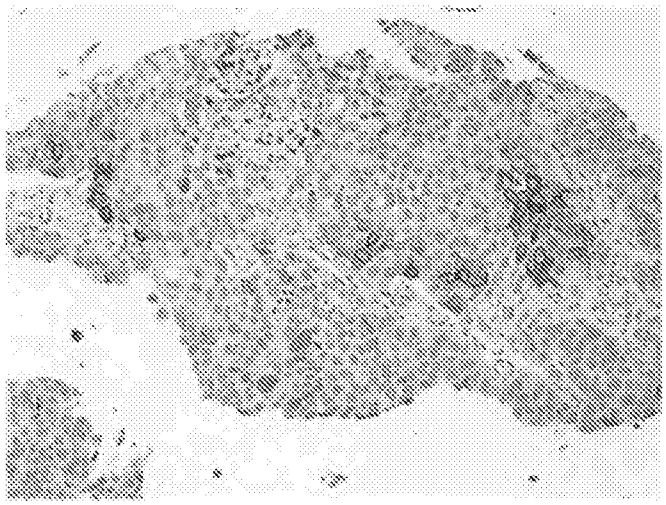

FIG. 4

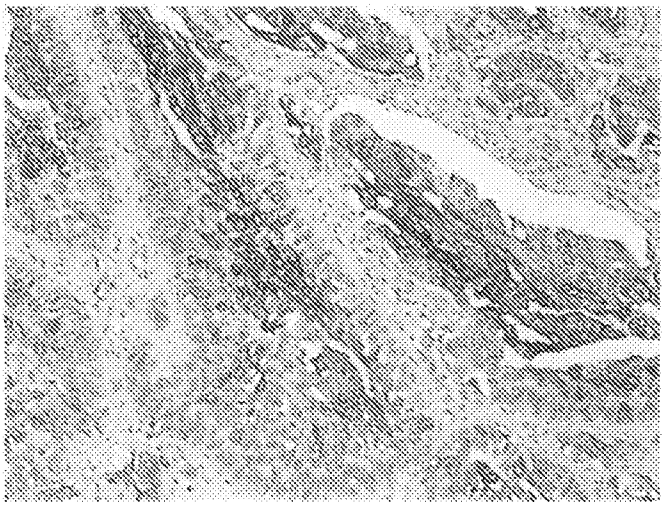

FIG. 5

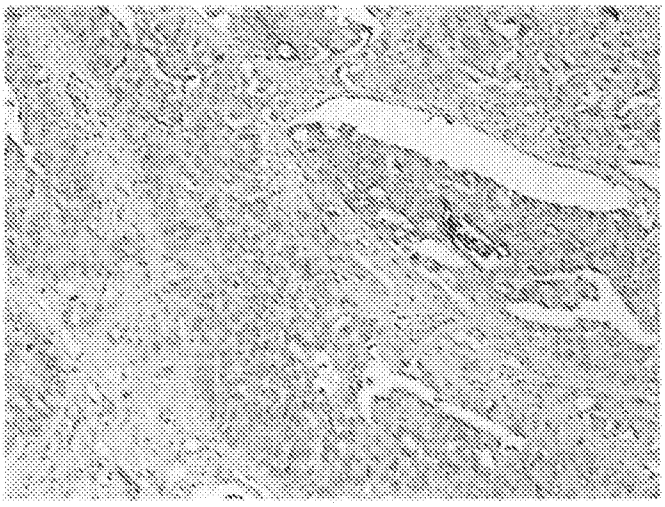

FIG. 6

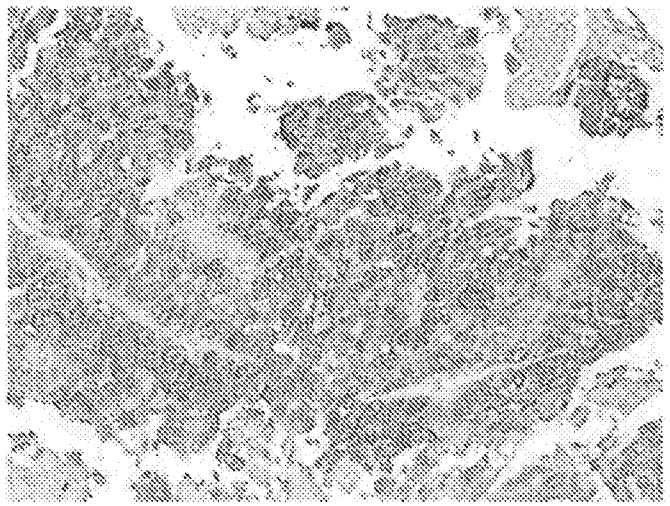

FIG. 7

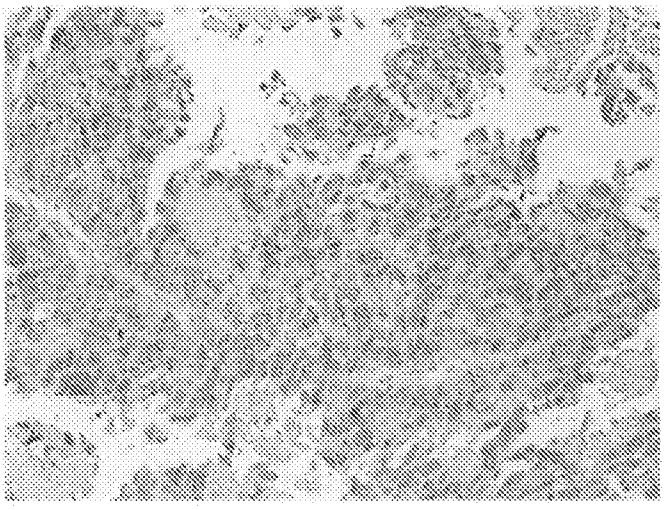

FIG. 8

Probed back with Purified Ms x UP II
@ 1 ug/ml
kDa
250
150
100
75
50
37
25
20
15
10
UP II Protein
0.002ug/lane
0.001ug/lane
0.0005ug/lane
0.0003ug/lane
UPK3a Protein
0.002ug/lane
0.001ug/lane
0.0005ug/lane
0.0003ug/lane

Fig. 9A

Probed back with Purified Ms x UPK3a
@ 1 ug/ml
kDa
250
150
100
75
50
37
25
20
15
10
UP II Protein
0.002ug/lane
0.001ug/lane
0.0005ug/lane
0.0003ug/lane
UPK3a Protein
0.002ug/lane
0.001ug/lane
0.0005ug/lane
0.0003ug/lane

Fig. 9B

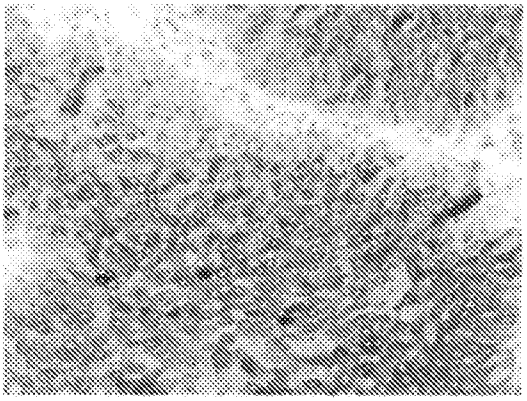

FIG. 10

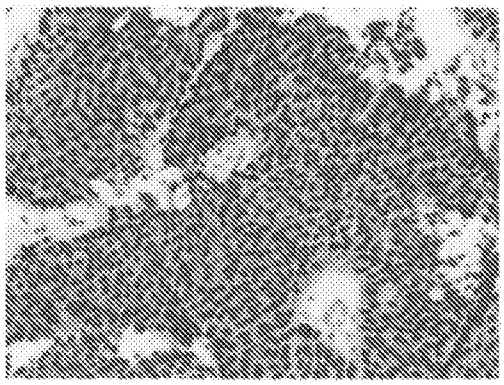

FIG. 11

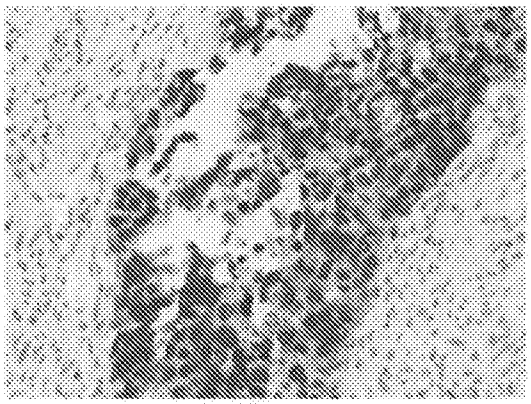

FIG. 12

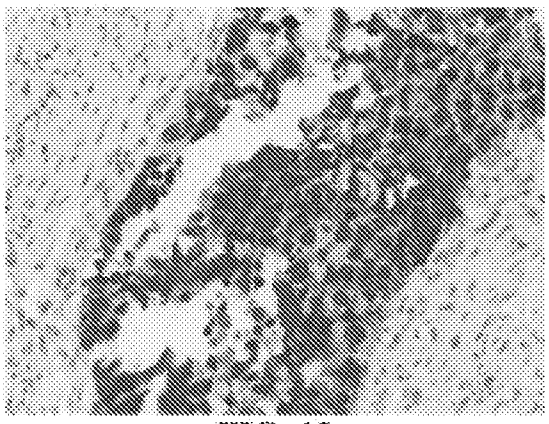

FIG. 13

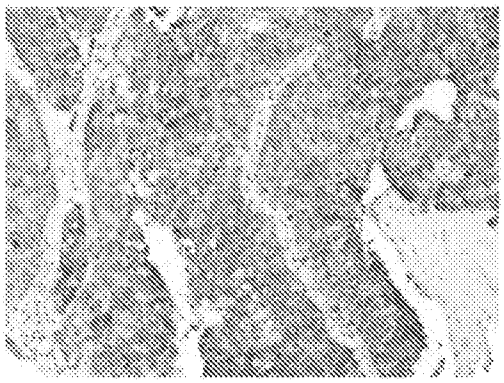

FIG. 14

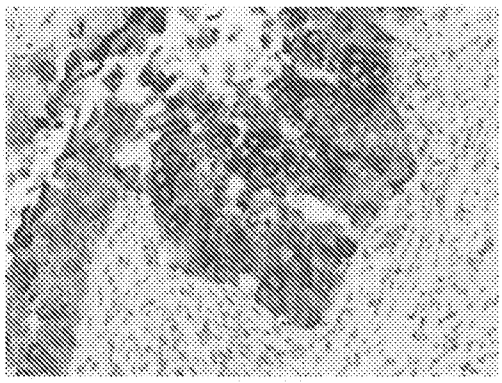

FIG. 15

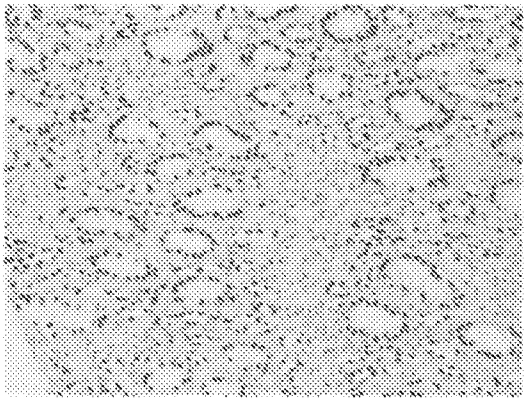

FIG. 16

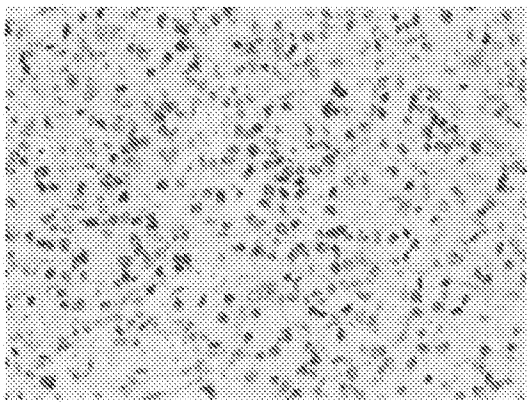

FIG. 17

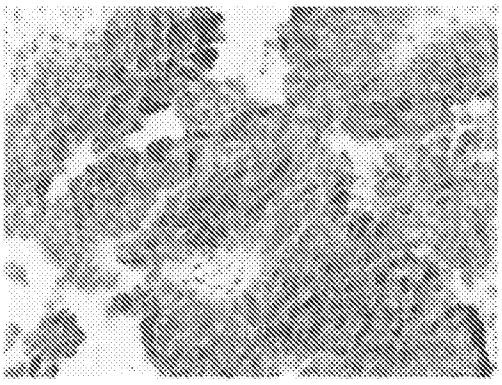

FIG. 18

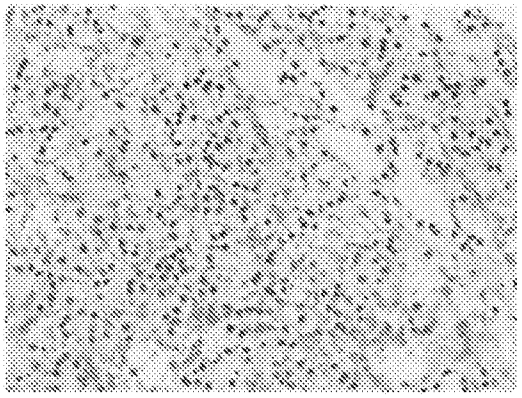

FIG. 19

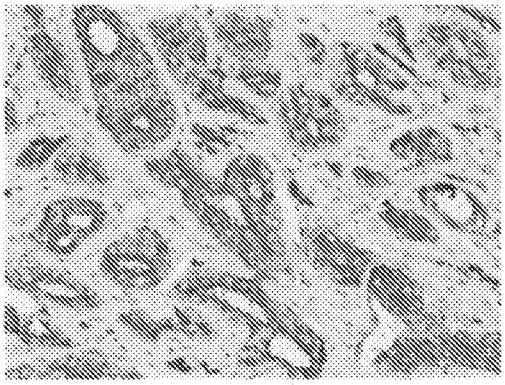

FIG. 20

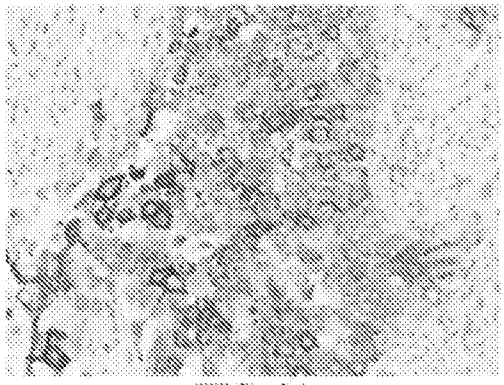

FIG. 21

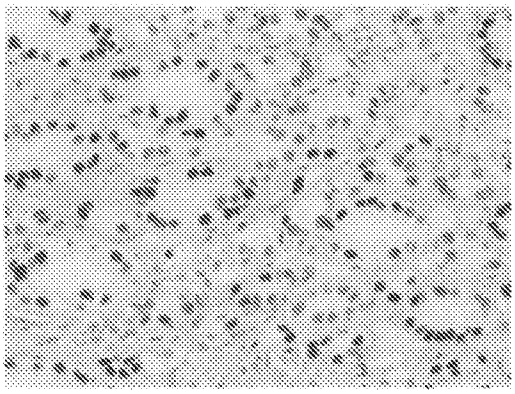

FIG. 22

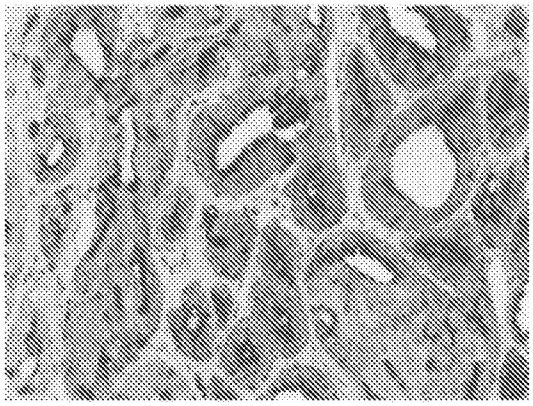

FIG. 23

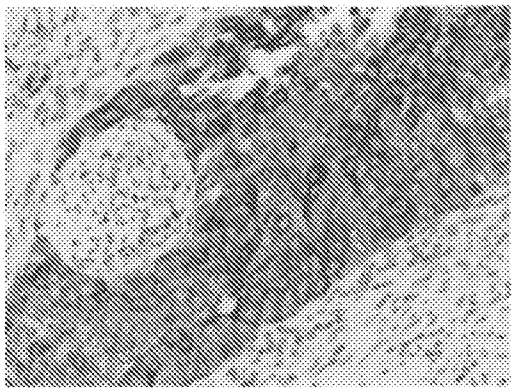

FIG. 24

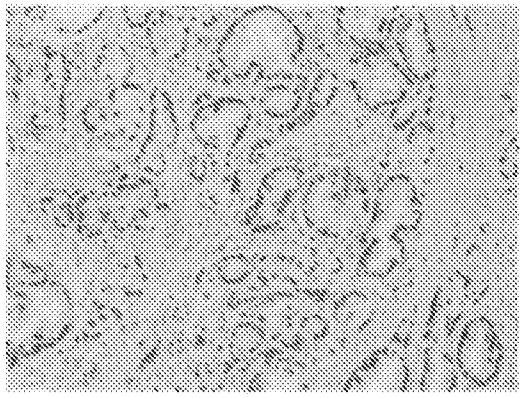

FIG. 25

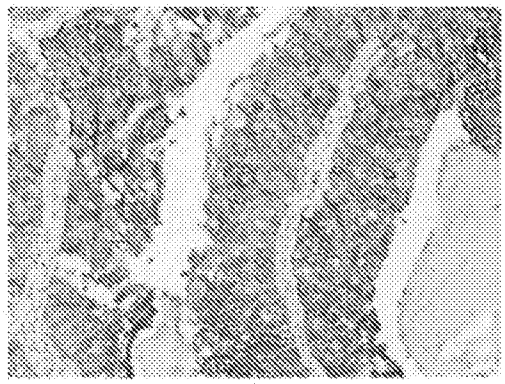

FIG. 26

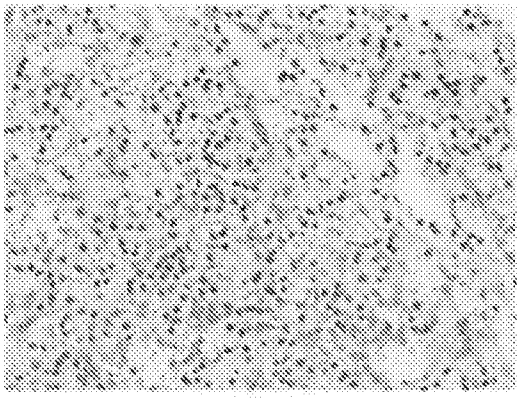

FIG. 27

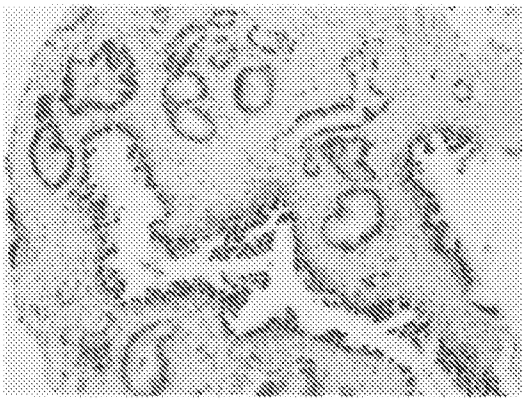

FIG. 28

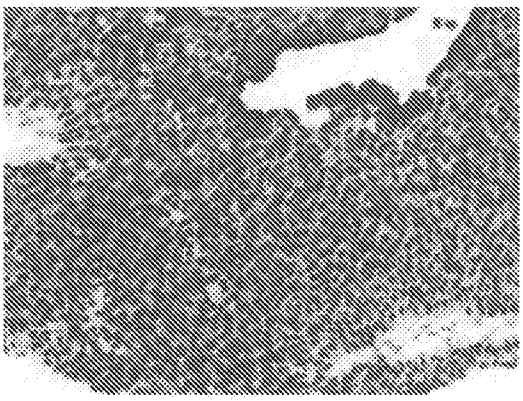

FIG. 29

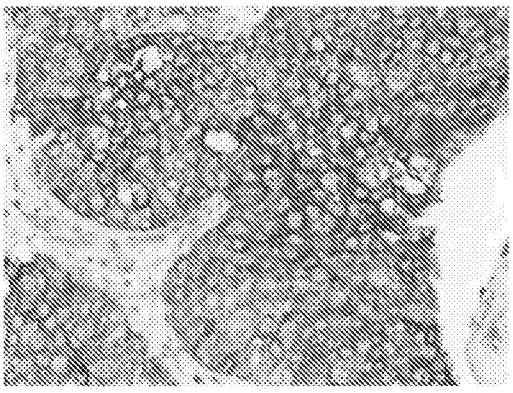

FIG. 30

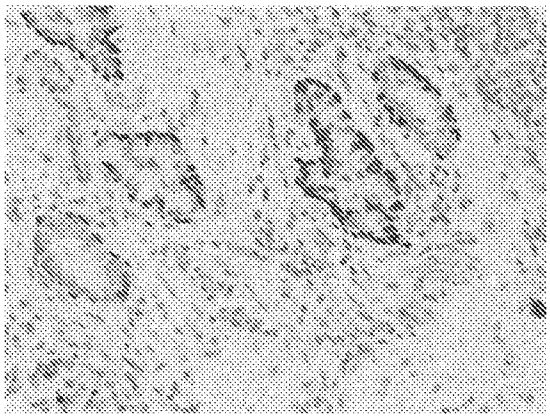

FIG. 31

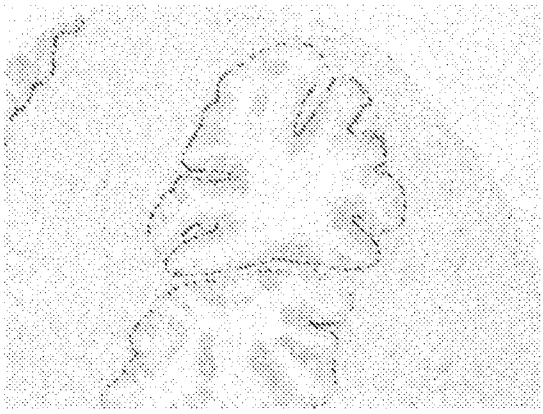

FIG. 32

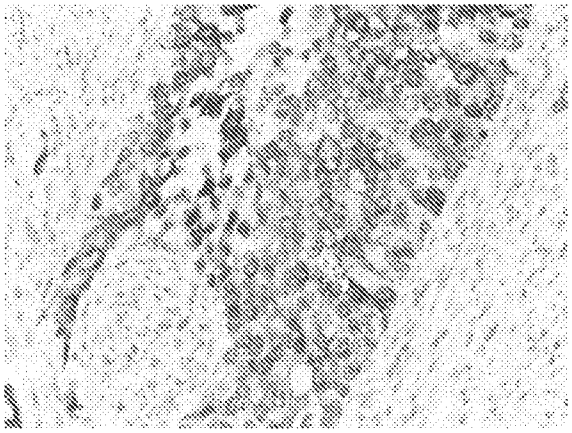

FIG. 33

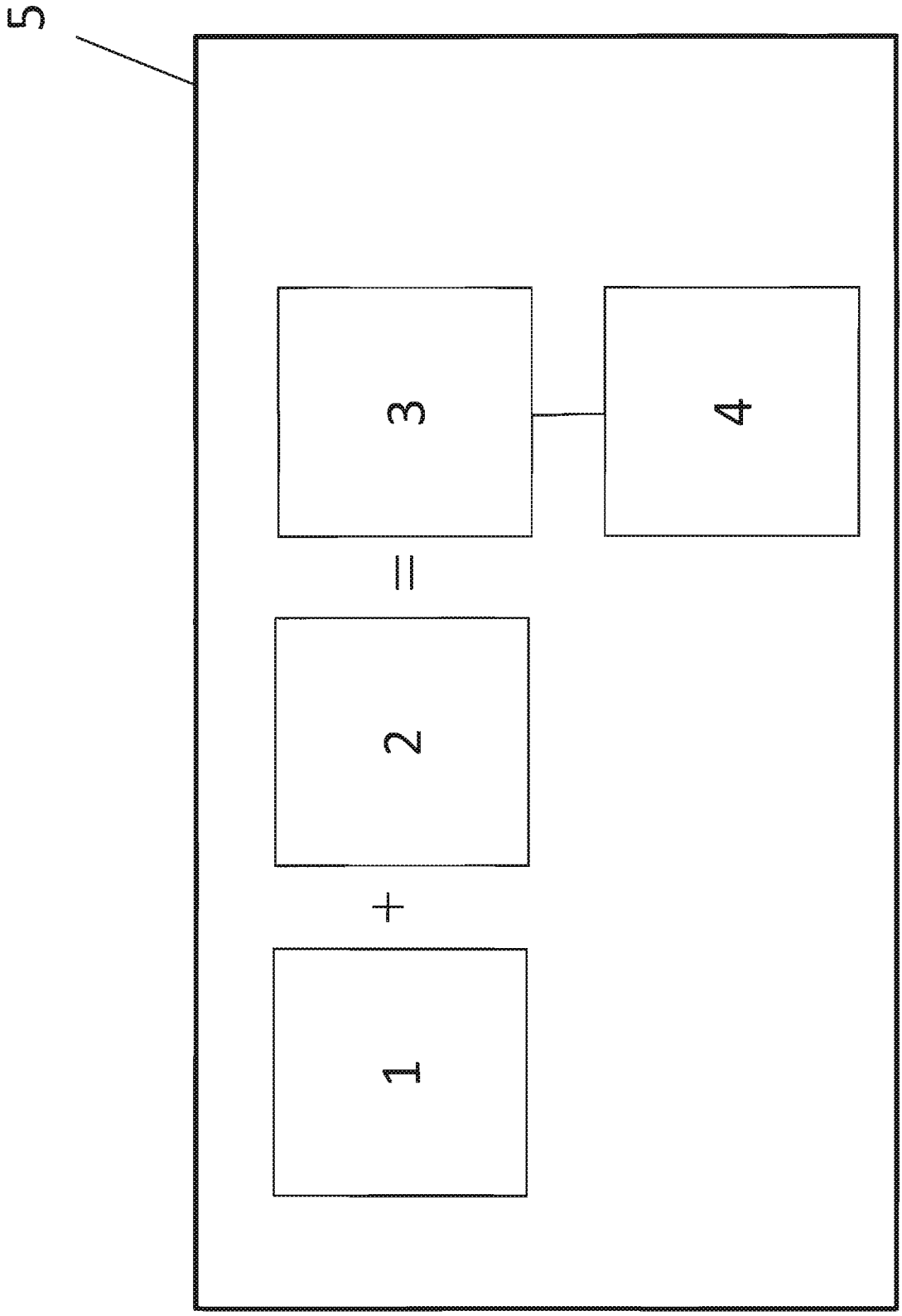

Fig. 34

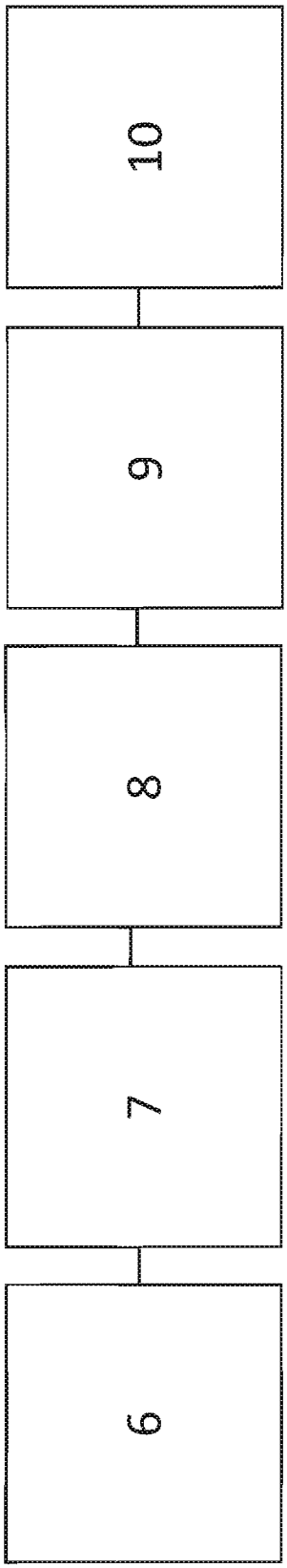

Fig. 35

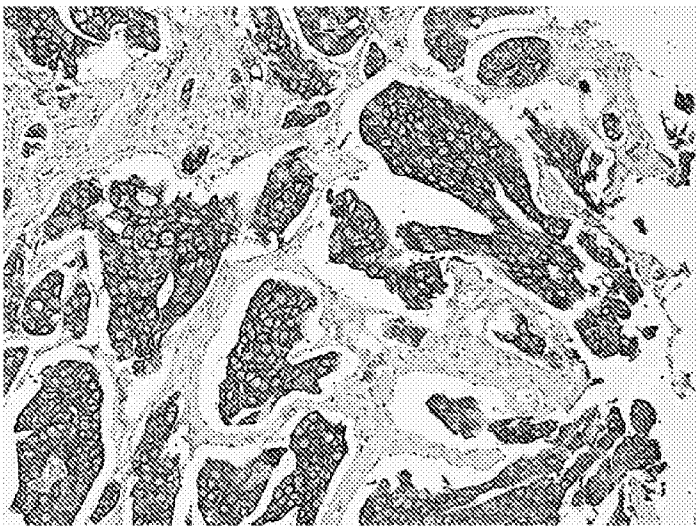

FIG. 36

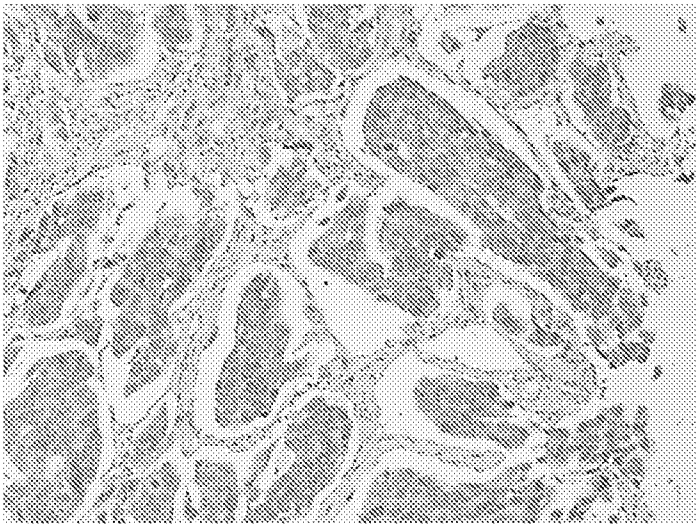

FIG. 37

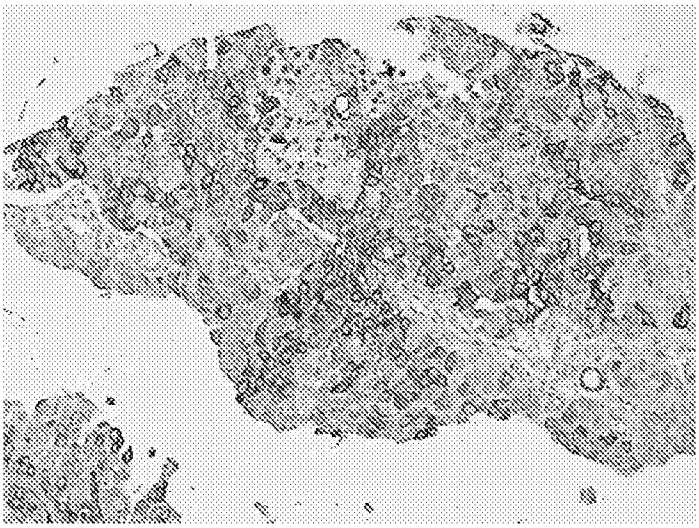

FIG. 38

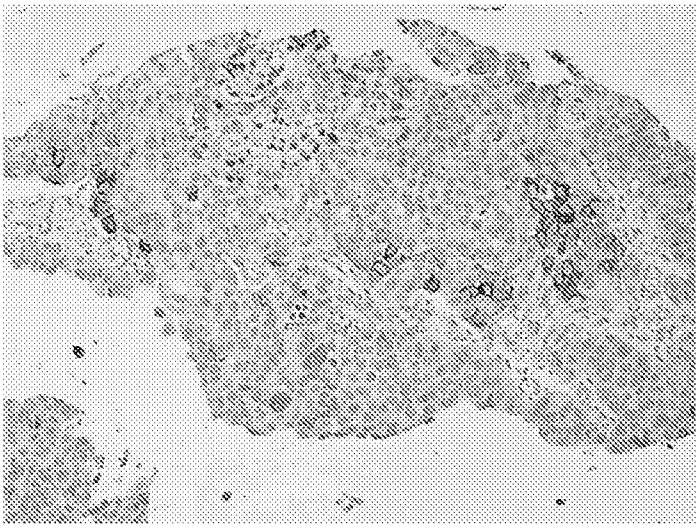

FIG. 39

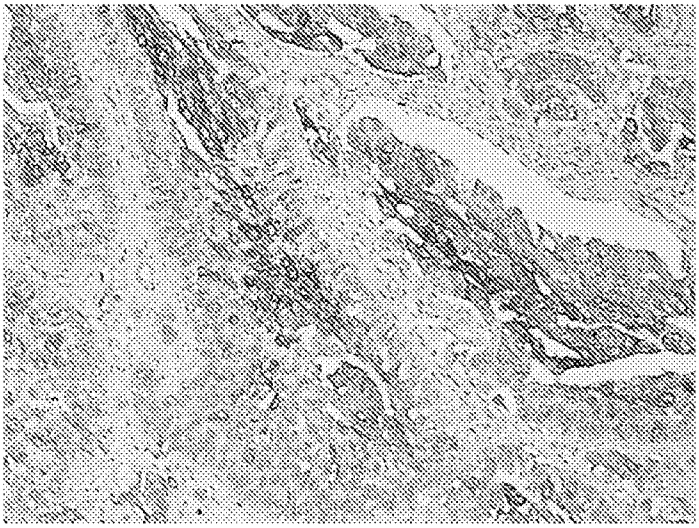

FIG. 40

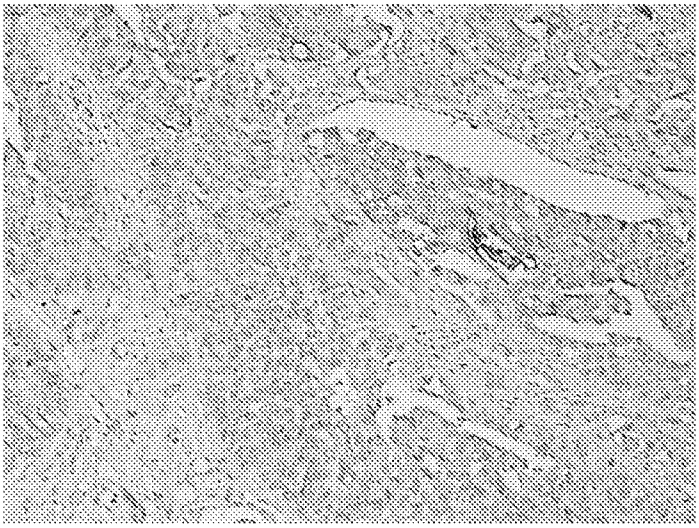

FIG. 41

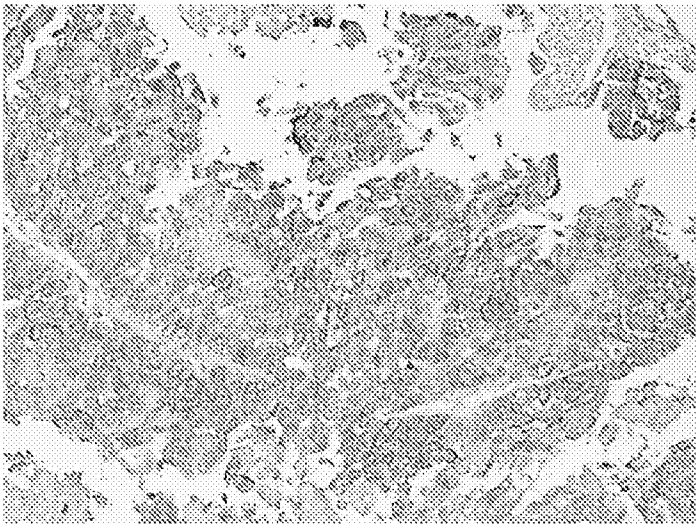

FIG. 42

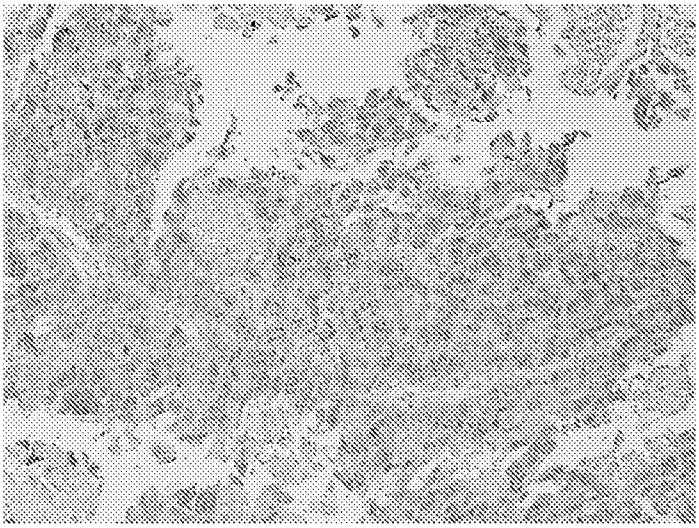

FIG. 43

FIG. 44A

FIG. 44B

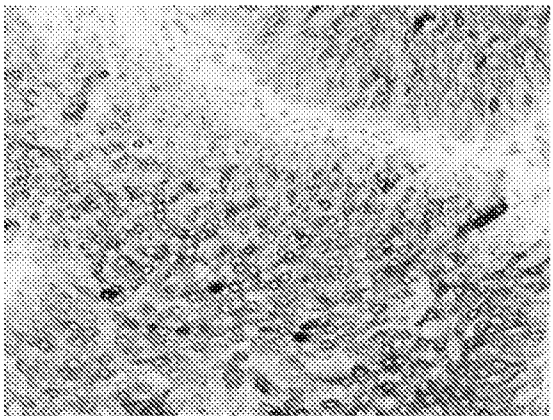

FIG. 45

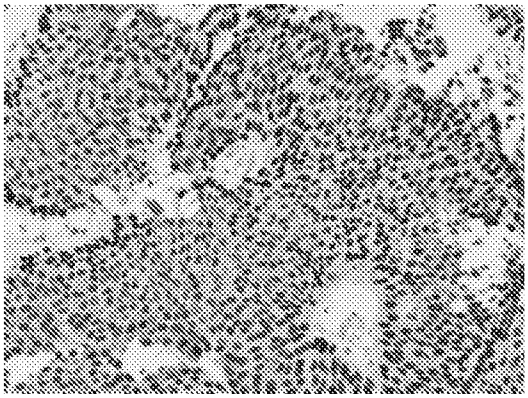

FIG. 46

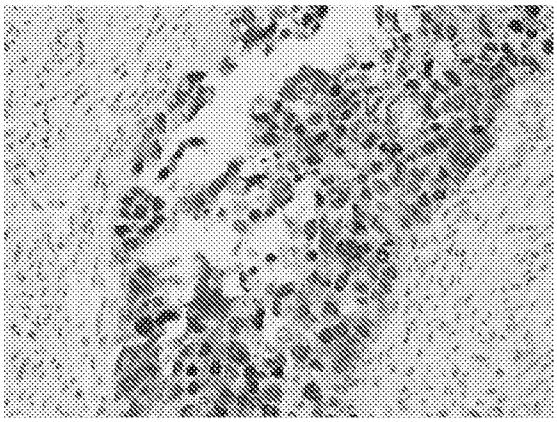

FIG. 47

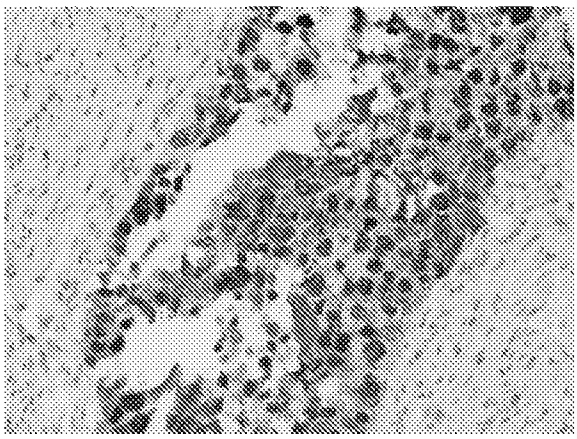

FIG. 48

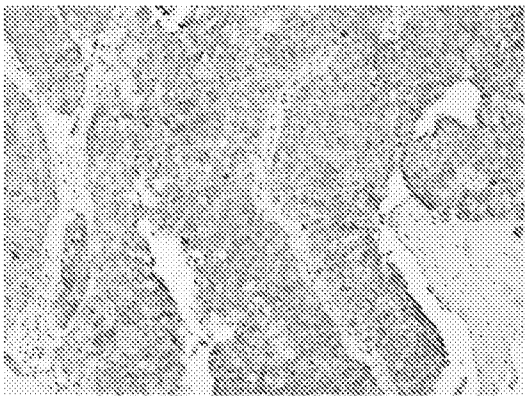

FIG. 49

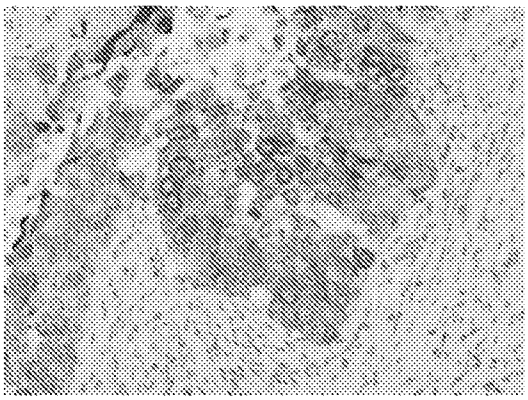

FIG. 50

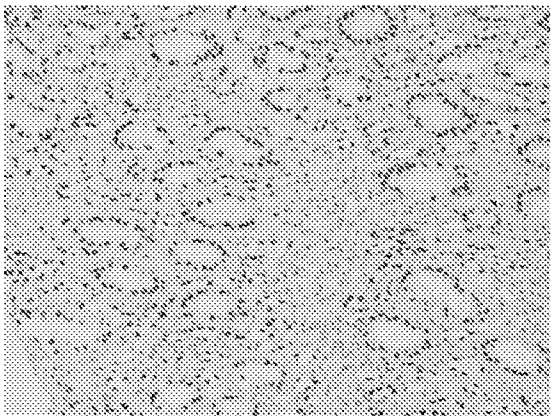

FIG. 51

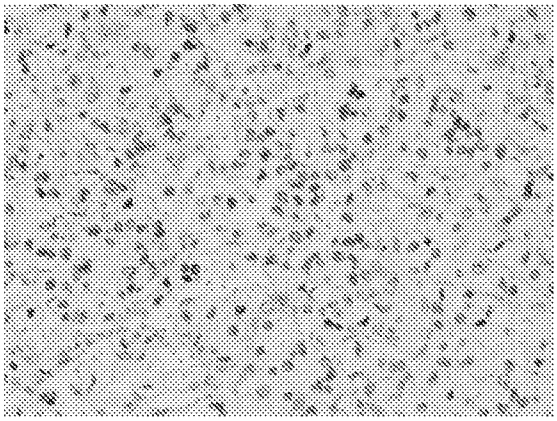

FIG. 52

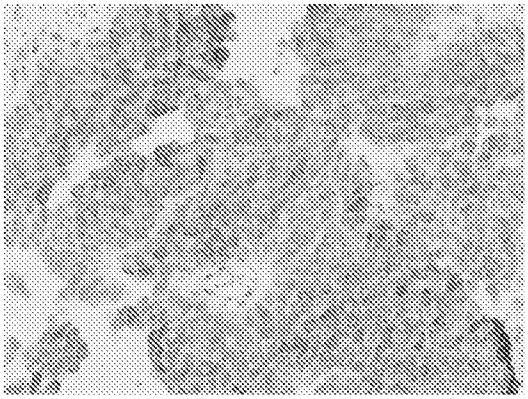

FIG. 53

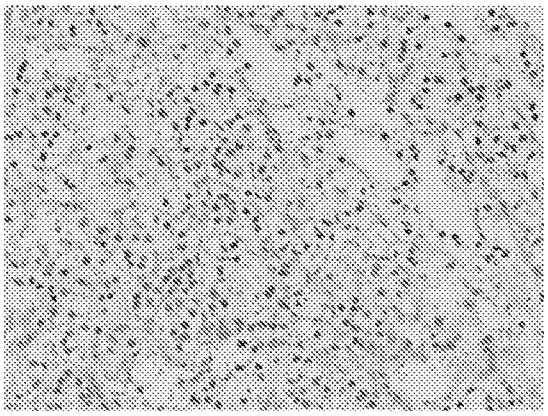

FIG. 54

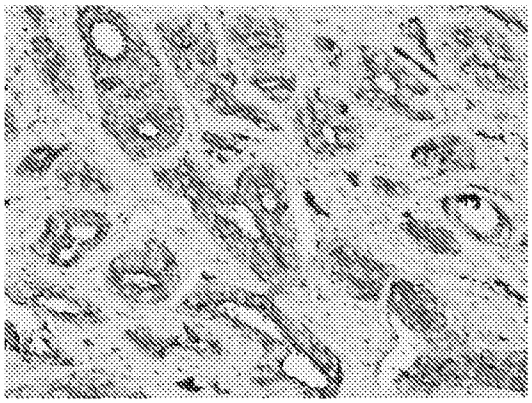

FIG. 55

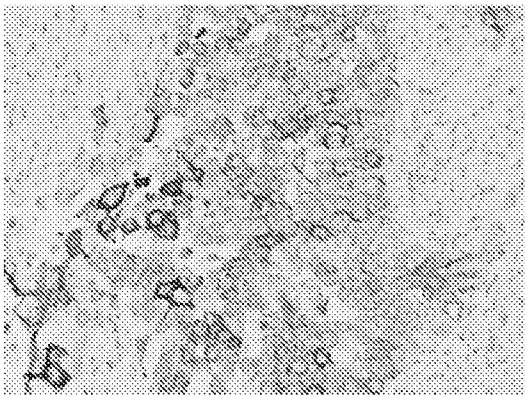

FIG. 56

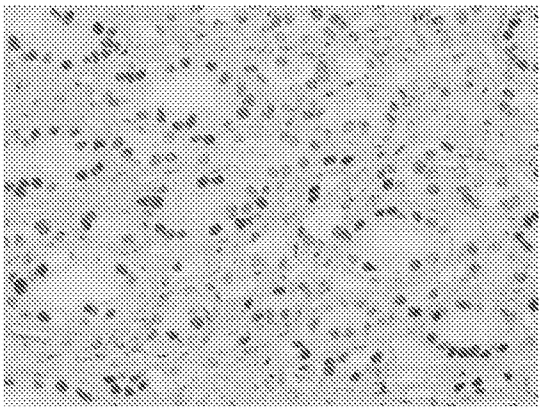

FIG. 57

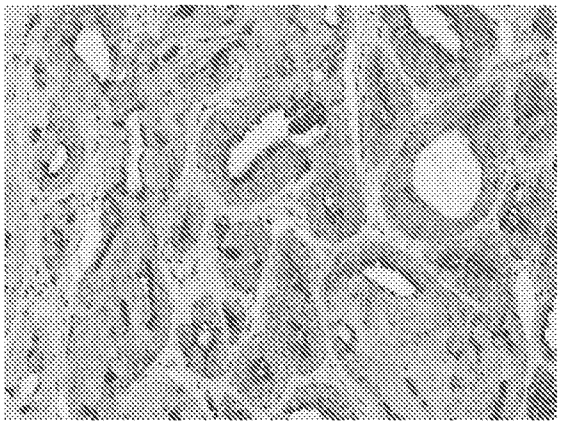

FIG. 58

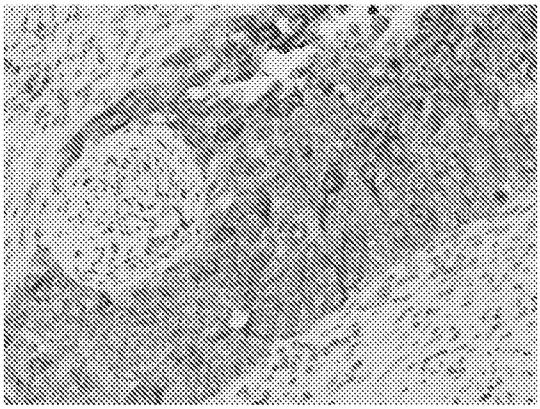

FIG. 59

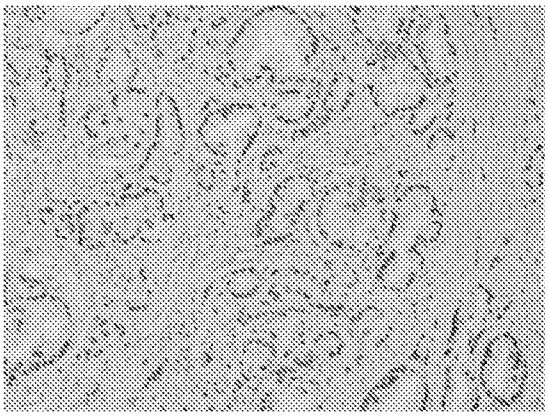

FIG. 60

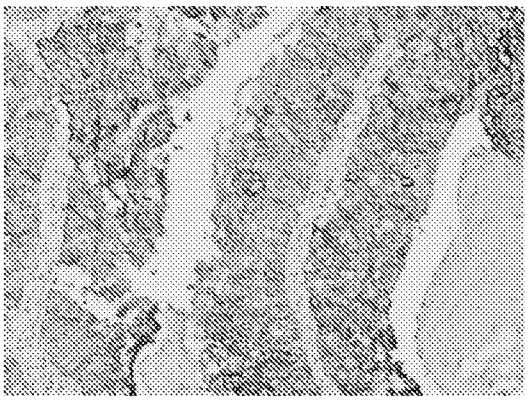

FIG. 61

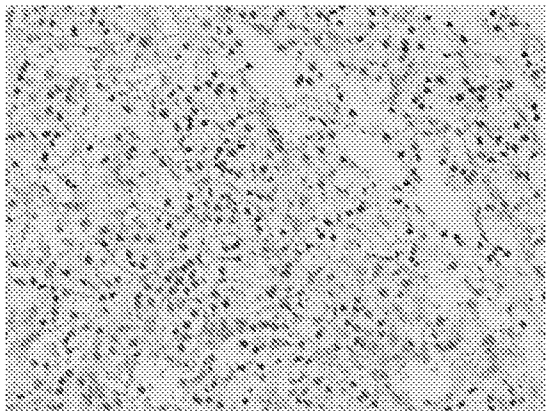

FIG. 62

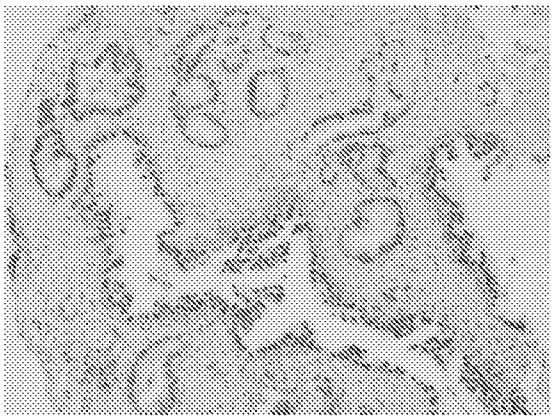

FIG. 63

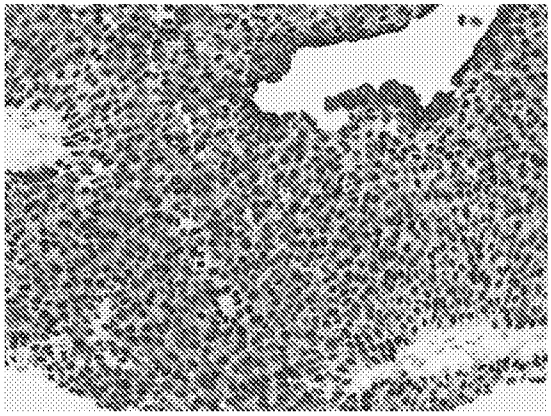

FIG. 64

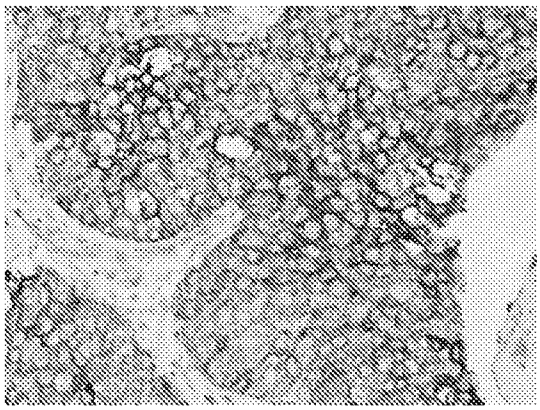

FIG. 65

FIG. 66

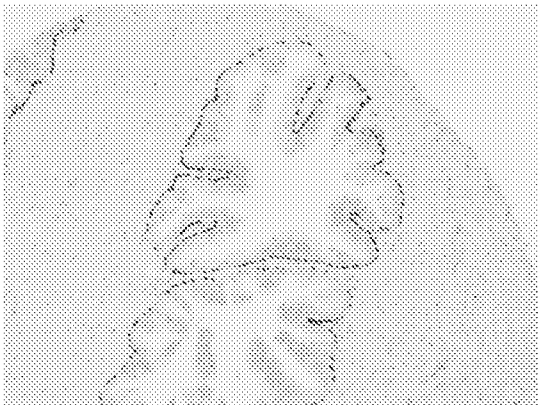

FIG. 67

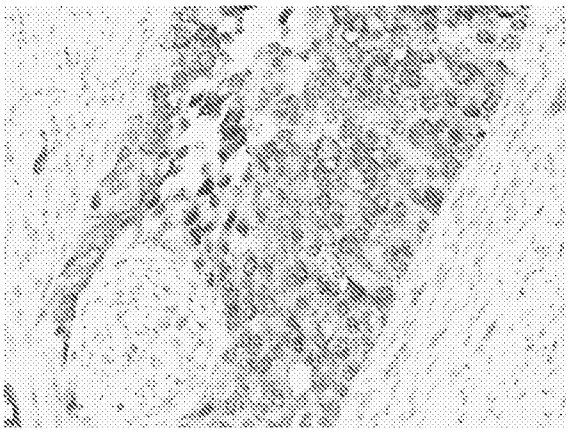

FIG. 68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62043

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; C07K 16/28, 16/30 (2014.01)
USPC - 435/6.14, 7.1, 6.1, 4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 39/395; C07K 16/28, 16/30; C12Q 1/68; G01N 33/53 (2014.01)
USPC: 435/6.14, 7.1, 6.1, 4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-Granted, US-Applications, EP-A, EP-B, WO, JP, DE-G, DE-A, DE-T, DE-U, GB-A, FR-A); ScienceDirect; Pubmed; Google;/Google Scholar; Search terms: 'Uroplakin II', antibody, fragment, cancer, 'bladder cancer', immunohistochemistry, 'positive indication cut-off', 'p63', 'Uroplakin III', 'PAX8'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	WO 2012/031273 A2 (DYLLA, SJ et al.) March 8, 2012; figure 10Q	7, 8 ----- 9/7, 9/8, 10/9/7, 10/9/8, 16, 17, 36/11, 36/31, 37/11, 37/31, 47/11, 47/12, 47/31, 48/11, 48/12, 48/31, 53/11, 53/12, 53/31, 54/53/11, 54/53/12, 54/53/31, 57/11, 57/31,78, 79 --- 33/5, 33/6, 38/5, 39/5, 40/39/5, 41/5, 41/6, 42/41/5, 42/41/6, 43/42/41/5, 43/42/41/6, 44/41/5, 44/41/6, 45/5, 45/6, 46/45/5, 46/45/6, 47/5, 47/6, 48/5, 48/6, 49/5, 49/6, 50/5, 50/6, 51/49/5, 51/49/6, 52/50/5, 52/50/6, 53/5, 53/6, 54/53/5, 54/53/6, 55/5, 55/6, 56/5, 56/6, 57/5, 57/6, 58/5, 58/6, 59/5, 59/6, 60/5, 60/6, 61/56/5, -continued on next page..

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2014 (10.01.2014)	29 JAN 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62043

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/031273 A2 (DYLLA, SJ et al.) March 8, 2012; figure 10Q	...61/56/6, 62/56/5, 62/56/6, 63/56/5, 63/56/6, 64/56/5, 64/56/6, 65/5, 65/6, 66/65/5, 66/65/6, 67/65/5, 67/65/6, 68/65/5, 68/65/6, 69/65/5, 69/65/6, 70/65/5, 70/65/6, 71/65/5, 71/65/6, 72/65/5, 72/65/6, 92/5, 92/6, 93/92/5, 93/92/6, 94/93/92/5, 94/93/92/6, 95/92/5, 95/92/6
X --- Y	WU, RL et al. Uroplakin II Gene Is Expressed In Transitional Cell Carcinoma But Not In Bilharzial Bladder Squamous Cell Carcinoma: Alternative Pathways Of Bladder Epithelial Differentiation And Tumor Formation. Cancer Research, 15 March 1998, Vol. 58, No. 6, pp 1291-1297; page 1292, right column, sixth paragraph to page 1293, left column, first paragraph; page 1293, figures 1, 2.	11, 12, 18-20, 28-30, 41/11, 41/12, 44/41/11, 44/41/12, 45/11, 45/12, 46/45/11, 46/45/12, 49/11, 49/12, 50/11, 50/12, 51/49/11, 51/49/12, 52/50/11, 52/50/12, 55/11, 56/11, 62/56/11, 65/11, 65/12, 66/65/11, 66/65/12, 67/65/11, 67/65/12, 69/65/11, 69/65/12, 72/65/11, 72/65/12, 73, 76, 80-82, 90, 91, 92/11, 92/12, 93/92/11, 93/92/12, 95/92/11, 95/92/12 --- 13, 14, 16, 17, 22-27, 31, 32, 36/11, 36/31, 37/11, 37/31, 38/31, 39/31, 40/39/31, 41/31, 42/41/11, 42/41/12, 42/41/31, 43/42/41/11, 43/42/41/12, 43/42/41/31, 44/41/31, 45/31, 46/45/31, 47/11, 47/12, 47/31, 48/11, 48/12, 48/31, 49/31, 50/31, 51/49/31, 52/50/31, 53/11, 53/12, 53/31, 54/53/11, 54/53/12, 54/53/31, 56/31, 57/11, 57/31, 58/11, 58/31, 59/11, 59/31, 60/11, 60/31, 61/56/11, 61/56/31, 62/56/31, 63/56/11, 63/56/31, 64/56/11, 64/56/31, 65/31, 66/65/31, 67/65/31, 68/65/11, 68/65/12, 68/65/31, 69/65/31, 70/65/11, 70/65/12, 70/65/31, 71/65/11, 71/65/12, 71/65/31, 72/65/31, 74, 75, 78, 79, 84-89, 92/31, 93/92/31, 94/93/92/11, 94/93/92/12, 94/93/92/31, 95/92/31
X	WO 2003/003906 A2 (MACK, DH et al.) January 16, 2003; abstract; page 4, lines 26-30	96
Y	WO 2010/022736 A2 (PEDERSEN, M et al.) March 4, 2010; page 129, lines 40-50	9/7, 9/8, 10/9/7, 10/9/8,16, 17, 37/11, 37/31, 78, 79

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/62043

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAUFMANN, O et al. Uroplakin III Is A Highly Specific And Moderately Sensitive Immunohistochemical Marker For Primary And Metastatic Urothelial Carcinomas. Am J Clin Pathol. May 2000, Vol. 113, No. 5, pp 683-687; page 684, right column, fourth paragraph	13, 14, 31, 32, 36/31, 37/31, 38/31, 39/31, 40/39/31, 41/31, 42/41/11, 42/41/12, 42/41/31, 43/42/41/11, 43/42/41/12, 43/42/41/31, 44/41/31, 45/31, 46/45/31, 47/31, 48/31, 49/31, 50/31, 51/49/31, 52/50/31, 53/31, 54/53/31, 56/31, 57/31, 58/31, 59/31, 60/31, 61/56/31, 62/56/31, 63/56/31, 64/56/31, 65/31, 66/65/31, 67/65/31, 68/65/31, 69/65/31, 70/65/31, 71/65/31, 72/65/31, 74, 75, 92/31, 93/92/31, 94/93/92/11, 94/93/92/12, 94/93/92/31, 95/92/31
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Y	WO 2010/124689 A1 (LIAO, Z et al.) November 4, 2010; page 6, lines 17-25	58/11, 58/31, 59/11, 59/31, 60/11, 60/31, 61/56/11, 61/56/31, 63/56/11, 63/56/31, 64/56/11, 64/56/31, 68/65/11, 68/65/12, 68/65/31, 70/65/11, 70/65/12, 70/65/31, 71/65/11, 71/65/12, 71/65/31