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Reproducible and highly sensitive detection of the broad spectrum epithelial marker keratin 19 in routine cancer diagnosis

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Aims: In this study the recently developed keratin 19 antibody RCK108 is biochemically and immunohistochemically characterized. Its applicability as a keratin marker in routinely processed histological tissue specimens is assessed.

Methods and results: The keratin 19 antibody RCK108 antibody was tested on normal and malignant routinely formalin-fixed, paraffin-embedded tissue specimens. It stains most, although not all, glandular epithelia and showed (focal) reactivity in the basal cell compartment of stratified epithelia. It was found to react with most epithelial tumours, including adenocarcinomas, squamous cell carcinomas and endocrine tumours of various origins.

Conclusions: Its reproducible and highly sensitive staining characteristics make RCK108 a useful antibody to be applied as a broad epithelial marker for carcinoma detection in routinely processed paraffin sections. As such, RCK108 is a specific reagent for practically all epithelial tumours. A few types of epithelial malignancies, known not to contain keratin 19, were negative for RCK108. Therefore the antibody is also useful in some narrow differential diagnostic considerations such as cholangiocellular carcinoma (RCK108 positive) vs. hepatocellular carcinoma (RCK108 negative). Another important feature of this antibody is that it shows very little reactivity in mesenchymal tissues, or mesenchymally derived tumours, as is frequently described for other keratin antibodies. A few leiomyosarcomas showed sporadic reactivity.

Keywords: antigen retrieval, carcinoma diagnosis, immunohistochemistry, keratin 19, RCK108

Introduction

Human keratins comprise a family of ≈30 different members, found almost exclusively in epithelial tissues. Of these, 20 have been found in the 'soft' epithelia, while others belong to the 'hard' keratins of nail and hair. Depending on molecular weight and isoelectric pH, the former keratin subtypes have been classified from 1 to 20 according to the Moll catalogue[1,2]. Keratins have been divided into two categories, i.e. the type I keratins (neutral or basic; comprising keratins 9 through 20) and the type II keratins (acidic; comprising keratins 1 through 8), which form heteropolymers. Based on gel electrophoresis and immunohistochemical tissue distribution studies, the keratin content in both normal and neoplastic tissues has been determined to a large extent[1–4]. These studies demonstrate that the distribution of individual keratins in epithelial tissues is not random, but complies to certain rules[1]. The keratin content of glandular epithelia, for example, comprises combinations of keratins 7, 8, 18, 19, or 20, depending

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on the tissue type in which they occur. More complex epithelia, such as stratified epithelia or combined epithelia, have a more complex keratin composition. In general, such stratifying tissues do not contain the glandular type keratins, but express, amongst others, keratins 1, 4, 10, 13 or 16, depending on their level of differentiation. In this respect, keratin 19 has an intermediate position, being observed in both glandular and stratified epithelia, albeit in the basal cell compartment of the latter. The presence of keratin 19 in normal cells has been related to functional growth, keeping post-stem cells in a flexible state of differentiation[5–7]. As cells differentiate, the appropriate epithelium-specific type I keratin can be exchanged with keratin 19[7]. This hypothesis may explain the ubiquitous presence of keratin 19 in proliferative compartments of epithelia. This may also explain the widespread expression of keratin 19 in premalignant and malignant cells[7].

A diagnostically important aspect of keratins is that their tissue-specific expression pattern is retained during epithelial malignant transformation. Also, the keratin phenotype of a metastatic carcinoma may be indicative of the primary location of the carcinoma[1–4]. One of the major drawbacks in the applicability of monoclonal keratin antibodies is that they often lack reactivity in formalin-fixed, paraffin-embedded tissue[4,8]. Even after considerable modifications[8] to staining protocols, many keratin antibodies remain unreactive. However, enhancement procedures based on proteolytic pre-treatment[8] or microwave-based antigen retrieval techniques[9] have been shown to result in a remarkable increase in immunoreactivity. We describe the development and characterization of a new monoclonal antibody to keratin 19 that displays a sensitive and specific reaction in the detection of carcinomas in formalin-fixed, paraffin-embedded tissues after microwave oven pre-treatment.

Materials and methods

PREPARATION OF ANTIBODY RCK108

The mouse monoclonal antibody producing clone RCK108 was obtained after fusion of SP2/0-Ag14 myeloma cells with splenic lymphocytes from a BALB/c mouse, immunized intraperitoneally with a cytoskeletal preparation of T24 cells. Fusion was carried out in polyethylene glycol-4000 and hybrids were grown in 24-well clusters in Roswell Park Memorial Institute medium 1640 (Dutch modification) containing 15% fetal calf serum. The cells were incubated a few hours before adding hypoxanthine, aminopterin and thymidine to the medium. Hybridoma cultures were tested for antibody production 2 weeks later (see Results section). Antibody RCK 108 is of the IgG1 subtype and is available from BioGenex (San Ramon, USA), DAKO A/S (Glostrup, Denmark) and Euro-Diagnostica BV (Arnhem, The Netherlands).

GEL ELECTROPHORESIS AND IMMUNOBLOTTING

One- and two-dimensional polyacrylamide gel electrophoresis and immunoblotting assays of cytoskeletons, prepared from several cell lines by extraction with 1% Triton X-100 in phosphate-buffered saline (PBS), pH 7.4, containing 0.4 mM phenylmethyl sulphonyl fluoride, 1 mM EGTA and 1 mM EDTA, were performed essentially as described before[10]. The cell lines used for these studies were chosen on the basis of their known keratin expression patterns and included MCF-7, T24, RT4, HeLa, MR65, as well as the human squamous cell carcinoma cell line HaCaT. The immunoglobulin subtype of RCK108 (IgG, kappa) was determined by the mouse monoclonal antibody isotyping kit from HBT (purchased from Gibco BRL, Gathersburg, MD, USA). Other antibodies used in this study were LP2K (keratin 19), CK18–2 (keratin 18), M20 (keratin 8), and RCK105 (keratin 7). Antibodies M20, RCK105 and CK18–2 are available from Eurodiagnostica BV, Arnhem, The Netherlands.

TISSUES

Formalin-fixed, paraffin-embedded tissue blocks were taken from the files of the Department of Pathology of the Canisius Wilhelmina Hospital, Nijmegen, The Netherlands. They represented both benign and malignant tumours as well as normal epithelial tissues (see Table 1 and Table 2). All tissue fragments had been fixed in 4% buffered (PBS, pH 7.4) formalin for at least 24 h before processing through paraffin for routine diagnosis.

IMMUNOHISTOCHEMISTRY

Four-micrometre thick sections were cut, mounted on poly-L-lysine coated slides, and deparaffinized in xylene followed by an ethanol series. After antigen retrieval using the microwave procedure with distilled water (see below), the sections were incubated for 30 min at room temperature, with the RCK108 antibody in a dilution of 1:10–1:50. After three subsequent washing steps in PBS, the SuperSensitive StreptAvidin detection kit (BioGenex Laboratories, San Ramon, USA) was applied to the sections, according to the manufacturer's instructions. The enzyme label used in most cases,

was horseradish peroxidase in combination with 0.03% H_2O_2 as substrate and 3,3′-diaminobenzidine-HCl (DAB) as chromogen. In these cases the endogenous peroxidase activity was blocked by quenching for 15 min in 3% H_2O_2 in distilled water. In those cases where a nonspecific DAB reaction was to be expected (e.g. liver), or where a differentiation between the DAB reaction product and pigment (e.g. skin) might lead to interpretation difficulties, the alkaline phosphatase enzyme label was used in combination with 1% naphthol AS-MX as substrate and Fast Red in 0.1M Tris-HCl pH 8.2 as chromogen. The sections were counterstained with haematoxylin and mounted in the appropriate mounting medium.

Results

IDENTIFICATION OF THE RCK108 ANTIGEN

Antibody RCK108 was initially selected on the basis of its reactivity with filamentous structures in cultured epithelial cells. The immunofluorescence staining pattern in T24 cells suggested reactivity with one of the intermediate filament protein (IFP) types in these cells, i.e. keratin or vimentin. Testing of vimentin negative cells (MCF-7 and HaCaT) excluded this type of IFP as antigen. Immunohistochemical testing of a restricted set of human tissues showed that RCK108 was exclusively staining epithelia, providing further support for the assumption that the reagent recognizes keratin(s). When hepatocytes and human skin, tissues known not to contain keratin 19, were tested, RCK108 staining was negative. Tissues known to contain keratin 19 (in addition to other keratins) were all positive for RCK108. To further test the assumption that RCK108 recognizes keratin 19, cytoskeleton preparations from T24, RT4, and HaCaT cells were separated by one- and two- dimensional gel electrophoresis and immunoblotted. In all three cell lines, RCK108 recognized a single protein band of 40 kDa, comigrating with a band that was recognized by antibody LP2K, known to detect keratin 19 specifically (Figure 1a–g). Two-dimensional immunoblotting studies finally confirmed that RCK108 reacts exclusively with keratin 19. Figure 2 shows the immunoblots of both HaCaT cells (Figure 2a–d) and RT4 (Figure 2e–h), subsequently incubated with RCK108, keratin 18 antibody CK18–2, keratin 8 antibody M20 and the keratin 7 antibody RCK105. RCK108 recognized a single spot in the two-dimensional immunoblot, migrating at the correct keratin 19 position as compared to the other keratins.

REACTIVITY OF THE KERATIN 19 ANTIBODY RCK108 IN TISSUE SPECIMENS

In a pilot study, we tested the reactivity of the RCK108 antibody on paraffin sections with and without pretreatment of slides with a 0.1% pronase (Sigma, Deisenhofen, Germany, Pronase XIV) solution in PBS, incubated for 10 min at 37°C prior to the primary antibody incubation. Also, microwave oven based antigen retrieval procedures[9] were tested, incubating the slides in 0.01 M citrate buffer pH 6.0, 1% periodic acid in distilled water, or distilled water. The sections were placed in the test solution and microwaved for two cycles of 5 min each at 750 W, after which they were left in this solution for 20 min, subsequently rinsed in distilled water and PBS, and then used for immunostaining as described above. The tissues used in this pilot study comprised nine adenocarcinomas of the mammary gland, 10 carcinomas of the thyroid gland (papillary, follicular and medullary), six transitional cell carcinomas of the urinary bladder (grade II and III) and six adenocarcinomas of the stomach. Optimal and reproducible results were obtained after the microwave oven based antigen retrieval procedure with distilled water. This method was used for all further immunostaining assays.

The reactivity patterns of RCK108 in paraffin-embedded normal and malignant tissues are listed in

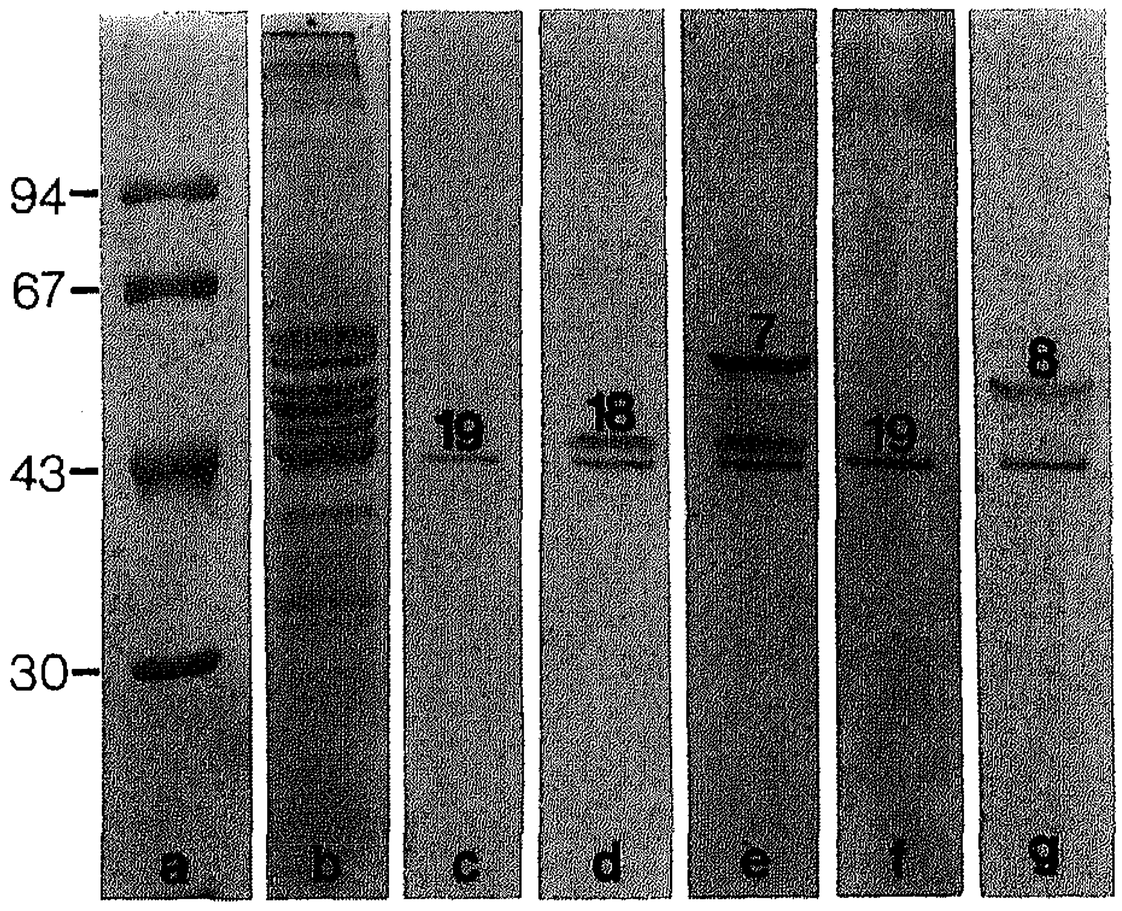


Figure 1. One-dimensional immunoblotting of a cytoskeletal preparation of HaCaT cells. **Lane a:** molecular weight marker proteins; **lane b:** total protein pattern of HaCaT cytoskeleton preparation; **lane c:** immunoblotting with RCK108; **lane d:** as lane c, but re-incubated with keratin 18 antibody CK18–2; **lane e:** as lane d, but re-incubated with keratin 7 antibody RCK105; **lane f:** immunoblotting with keratin 19 antibody LP2K; **lane g:** as lane f, but re-incubated with keratin 8 antibody M20. Keratins are indicated by their respective numbers.

Table 1. RCK108 staining reaction patterns in formalin-fixed normal human tissues

Tissue	Reactivity patterns
Skin	
Epidermis	
Basal layer	S/+
Superficial layers	−
Merkel cells	+
Sebaceous gland	++
Hair follicle	
Hair bulb	−
External root sheath	F/++
Internal root sheath	−
Eccrine sweat gland	
Secretory coil —clear cells	+++
—dark cells	+++
—myoepithelial cells	−
Secretory duct—luminal cells	++
—peripheral cells	−
Lip, non-keratinizing squamous epithelium	−
Tongue, non-keratinizing squamous epithelium	−
Larynx, non-keratinizing squamous epithelium	+++
Mesothelium	+++
Respiratory tract	
Lung	
Bronchi	
Columnar ciliated epithelial cells	+++
Basal cells	++
Alveoli	
Alveolar lining cells	+++
Gastrointestinal tract	
Salivary glands	
Parotid gland	
Secretory cells —cuboidal cells	+
—basal cells	−
Intercalated ducts —cuboidal cells	++
—basal cells	+++
Striated ducts —cuboidal cells	+++
—basal cells	+++
Intralobular ducts —cuboidal cells	+++
—basal cells	+++
Minor salivary gland	
Ductal epithelium	+++
Acinar epithelium	F/+++
Basal cells	+++
Oesophagus, non-keratinizing squamous epithelium—basal cells	F/+
—suprabasal cells	−
Stomach	
Antrum—mucous secreting cells	F/++
—gastric glands	++
Corpus —mucous secreting cells	++
—gastric glands	++
—neuroendocrine cells	++

Table 1 /continued....

Tissue		Reactivity patterns
Small intestine		
Villus epithelium		+++
Crypt epithelium		++
Brunner gland epithelium		–
Colon		
Epithelium		++
Pancreas		
Ductal epithelium		+++
Acinar epithelium		F/++
Islets of Langerhans		–
Liver		
Hepatocytes		–
Bile duct epithelium		+++
Gallbladder		
Epithelium		+++
Female genital tract		
Mammary gland		
lobules	—columnar cells	+++
	—myoepithelium	–
Ducts	—columnar cells	+++
	—myoepithelium	–
Cervix		
Endocervix	—columnar cells	++
	—reserve cells	+++
Ectocervix	—basal cells	++
	—suprabasal cells	–
Endometrium	—glandular epithelium	+++
Ovary		
Surface epithelium		–
Placenta		
Cytotrophoblast		+++
Male genital tract		
Prostate		
Ductal epithelium		+++
Basal cells		+++
Seminal vesicle		+++
Testis		
Sertoli cells/germ cells		–
Leydig cells		–
Rete testis		++
Urinary tract		
Kidney		
Epithelium of Bowman's capsule		F/+
Proximal tubular epithelium		++
Distal tubular epithelium		+++
Collecting duct epithelium		+++
Henle's loop epithelium		++
Urinary bladder		
Transitional epithelium		++

Table 1 /continued....

Tissue	Reactivity patterns
Endocrine tissue	
Thyroid gland	
Follicle epithelium	F/+
Parathyroid gland	
Epithelium	–
Adrenal gland	
Cortex	–
Miscellaneous	
Muscle tissue	
Skeletal muscle	–
Cardiac muscle	–
Smooth muscle	–
Vascular endothelium	–
Blood cells	
Erythrocytes	–
Lymphocytes	–
Peripheral nerve tissue	–
Fibroblasts	–
Bone tissue	
Osteocytes	–
Osteoblasts	–
Chondrocytes	–

Unless otherwise indicated, positive cases showed a reaction in 50% to 100% of the cells.
S, scattered cells are stained (less than 5%); F, focal staining pattern (5% to 50% of cells are stained); +, weak staining reaction; ++, moderate staining reaction; +++, strong staining reaction; –, negative.

Tables 1 & 2. As the tables give detailed information, we describe only the most salient features of the keratin 19 distribution patterns.

In normal epithelia, all columnar cells showed reactivity with RCK108 to a variable extent, with the exception of secretory cells of the parotid gland and Brunners' glands in the duodenum. Thyroid epithelium was focally and weakly positive for RCK 108. In liver, bile duct epithelium was strongly positive while hepatocytes were negative. Complex columnar epithelia, such as breast and eccrine sweat glands, showed intense staining of columnar cells lining the ducts, but the myoepithelium was negative for RCK 108. The myoepithelial cells adjacent to the secretory cells of the parotid gland were also negative. Prostatic basal cells however, were positive for keratin 19; basal cells of the minor salivary glands and basal cells lining intercalated, striated and interlobular ducts of the parotid were also positive for RCK 108. Reserve cells in the endocervix were all positive for RCK 108. Transitional epithelia lining the urinary tract showed intense staining through their full thickness. Muscle tissues, endothelial cells, bone marrow and fibroblasts were negative for RCK 108. The basal cell compartments of non-keratinizing squamous epithelia were usually positive for keratin 19. In the oral cavity focal staining, limited to the basal cell compartment, was observed. Epithelia lining tongue and lip were negative. In some cases the keratinizing squamous epithelium of the skin showed a weak and scattered expression of RCK108 in the basal cell layer, which may represent staining in Merkel cells.

When tested in epithelial malignancies, RCK 108 reactivity was detectable in all adenocarcinomas examined (Figure 3a,b). The antibody allowed a clear identification of adenocarcinoma cells dispersed throughout epithelium of Paget's disease (Figure 3c). Also the three main types of thyroid gland tumours (Figure 3d,e,f) were positive. As opposed to cholangiocellular carcinomas, which were all RCK 108 positive (Figure 3g), hepatocellular carcinomas were negative (Figure 3h). Neuroendocrine carcinomas of the pituitary gland (Figure 3i) and lung (Figure 3j) were positive, irrespective of their classification. Transitional cell carcinomas (Figure 3k) showed a strong reaction with the RCK108 antibody. Non-keratinizing squamous cell carcinomas of the lung, cervix

Table 2. RCK108 staining reaction patterns in formalin-fixed malignant human tissues

Tissue	Proportion of positive cases	Reactivity patterns
Skin		
Intradermal naevus	0/1	–
Compound naevus	0/1	–
Actinic keratosis	0/1	–
Bowen's disease	0/2	–
Basal cell carcinoma	4/4	F/+
Keratoacanthoma	1/1	F/+
Well-differentiated keratinizing squamous cell carcinoma	3/3	+
Poorly differentiated keratinizing squamous cell carcinoma	0/1	–
Melanoma	0/3	–
Lip		
Basal cell carcinoma	0/1	–
Well-differentiated non-keratinizing squamous cell carcinoma	1/3	F/+
Moderately differentiated squamous cell carcinoma	0/5	–
Tongue		
Well-differentiated squamous cell carcinoma	1/2	F/+
Moderately differentiated squamous cell carcinoma	1/5	+++
Poorly differentiated squamous cell carcinoma	1/1	F/+
Nasopharynx		
Papilloma; transitional epithelium	1/1	+++
Inverted papilloma; transitional epithelium	3/3	+++
Larynx		
Well-differentiated squamous cell carcinoma	1/3	F/+
Moderately differentiated squamous cell carcinoma	3/4	F/+
Poorly differentiated squamous cell carcinoma	1/1	F/+
Mesothelioma	2/2	+++
Respiratory tract		
Squamous cell carcinoma		
Moderately differentiated squamous cell carcinoma	6/6	+++
Poorly differentiated squamous cell carcinoma	1/1	+++
Adenocarcinoma		
Well-differentiated adenocarcinoma	1/1	+++
Moderately differentiated adenocarcinoma	2/2	+++
Poorly differentiated adenocarcinoma	1/1	+++
Adenosquamous carcinoma	1/1	+++
Small cell lung carcinoma	5/5	++
Gastrointestinal tract		
Parotid gland		
Pleomorphic adenoma	5/5	+++
Mucoepidermoid carcinoma		
Epidermoid component	1/1	++
Mucinous component	2/2	+++
Acinic cell carcinoma	1/1	+++
Oesophagus		
Well differentiated squamous cell carcinoma	3/3	+++
Moderately differentiated squamous cell carcinoma	3/3	+++
Stomach		
Adenocarcinoma	4/4	+++
Carcinoid	2/2	+++
Lymph node metastasis of gastric adenocarcinoma	1/1	+++
Small intestine		
Adenocarcinoma	1/1	++

Table 2 /continued....

Tissue	Proportion of positive cases	Reactivity patterns
Appendix		
Carcinoid	1/1	F/+
Colon		
Well-differentiated adenocarcinoma	2/2	+++
Moderately differentiated adenocarcinoma	9/9	+++
Lymph node metastasis of colon adenocarcinoma	2/2	+++
Carcinoid (goblet cell type)	1/1	++
Pancreas		
Well differentiated adenocarcinoma	7/7	+++
Liver		
Hepatocellular carcinoma	0/9	–
Cholangiolocellular carcinoma	6/6	+++
Mammary gland		
Ductal carcinoma	12/12	+++
Lobular carcinoma	4/4	+++
Combined ductal/lobular carcinoma	4/4	+++
Lymph node metastasis of ductal carcinoma	2/2	++
Paget's disease	2/2	++
Female genital tract		
Ovary		
Cystadenocarcinoma		
Serous type	5/5	+++
Mucinous type	6/6	+++
Endometroidcarcinoma	7/7	+++
Malignant Brenner tumour	1/1	+++
Clear cell carcinoma	2/2	+++
Cervix		
Squamous cell carcinoma		
Large cell non-keratinizing	2/2	++
Small cell non-keratinizing	2/2	+++
Adenocarcinoma		
Well differentiated	7/7	+++
Moderately differentiated	1/1	++
Poorly differentiated	4/4	+++
Adenosquamous carcinoma	8/8	+++
Clear cell carcinoma	2/2	+++
Endometrium		
Well-differentiated endometrioid carcinoma	9/9	+++
Poorly differentiated endometrioid carcinoma	4/4	++
Adenosquamous carcinoma	3/3	+++
Vulva		
Well-differentiated squamous cell carcinoma	5/5	F/+
Moderately differentiated squamous cell carcinoma	1/4	F/+++
Male genital tract		
Prostate		
Well differentiated adenocarcinoma	4/4	+++
Moderately differentiated adenocarcinoma	3/3	++
Poorly differentiated adenocarcinoma	1/1	F/+
Testis		
Seminoma	0/7	–
Malignant teratoma, intermediate type	3/3	+++
Choriocarcinoma	2/2	+++

Table 2 /continued....

Tissue	Proportion of positive cases	Reactivity patterns
Embryonal cell carcinoma	0/1	–
Sertoli cell tumour	0/1	–
Urinary tract		
Kidney: renal cell carcinoma	6/6	++
Urinary bladder; transitional cell carcinoma (grades 2 & 3)	9/9	+++
Endocrine tissues		
Thyroid gland		
Papillary carcinoma	2/2	++
Follicular carcinoma	4/4	++
Medullary carcinoma	2/2	++
Adrenal gland		
Phaeochromocytoma	0/2	–
Pituitary gland		
Chromophobic adenoma	1/1	++
Acidophil adenoma	1/1	++
Neuronal epithelial tissues		
Astrocytoma (grades 2–4)	4/4	++
Neurofibroma	0/2	–
Meningioma	4/4	++
Miscellaneous		
Lymphoma		
Non-Hodgkin		
Centrocytic	0/2	–
Centroblastic	0/2	–
Hodgkin's disease	0/2	–
Nodular sclerosing	0/1	–
Chondrosarcoma	0/1	–
Osteosarcoma	0/1	–
Malignant fibrous histiocytoma	0/2	–
Synovial sarcoma	0/1	–
Lipoma	0/2	–
Fibroma	0/2	–
Leiomyosarcoma	5/13	S/++

Unless otherwise indicated, positive cases showed a reaction in 50% to 100% of the cells.
S, scattered cells are stained (less than 5%); F, focal staining pattern (5% to 50% of cells are stained); +, weak staining reaction; ++, moderate staining reaction; +++, strong staining reaction; –, negative.

(Figure 3l), vulva, oral cavity and oesophagus (Figure 3m) were positive with some variation in immunostaining intensity between the cases. Focal positivity was observed in three out of four keratinizing squamous cell carcinomas and in all basal cell carcinomas (Figure 3n). Benign lesions of the skin were negative. Pleomorphic adenomas of the salivary gland were strongly positive, with a negative basal cell component. In some testicular teratomas we noted reactivity in the epithelial component, while Sertoli cell tumours, seminomas and embryonal cell carcinomas were negative. Furthermore, phaeochromocytomas, lymphomas, chondrosarcomas and osteosarcomas were negative.

Of the non-epithelial malignancies, all gliomas showed strong focal positivity in tumour cells (Figure 3o). Some leiomyosarcomas showed immunoreactivity with the RCK108 antibody, the number of positive cells ranging from 1% to 10% staining with various staining intensities and one epithelioid leiomyosarcoma showing weak reactivity in virtually all tumour cells.

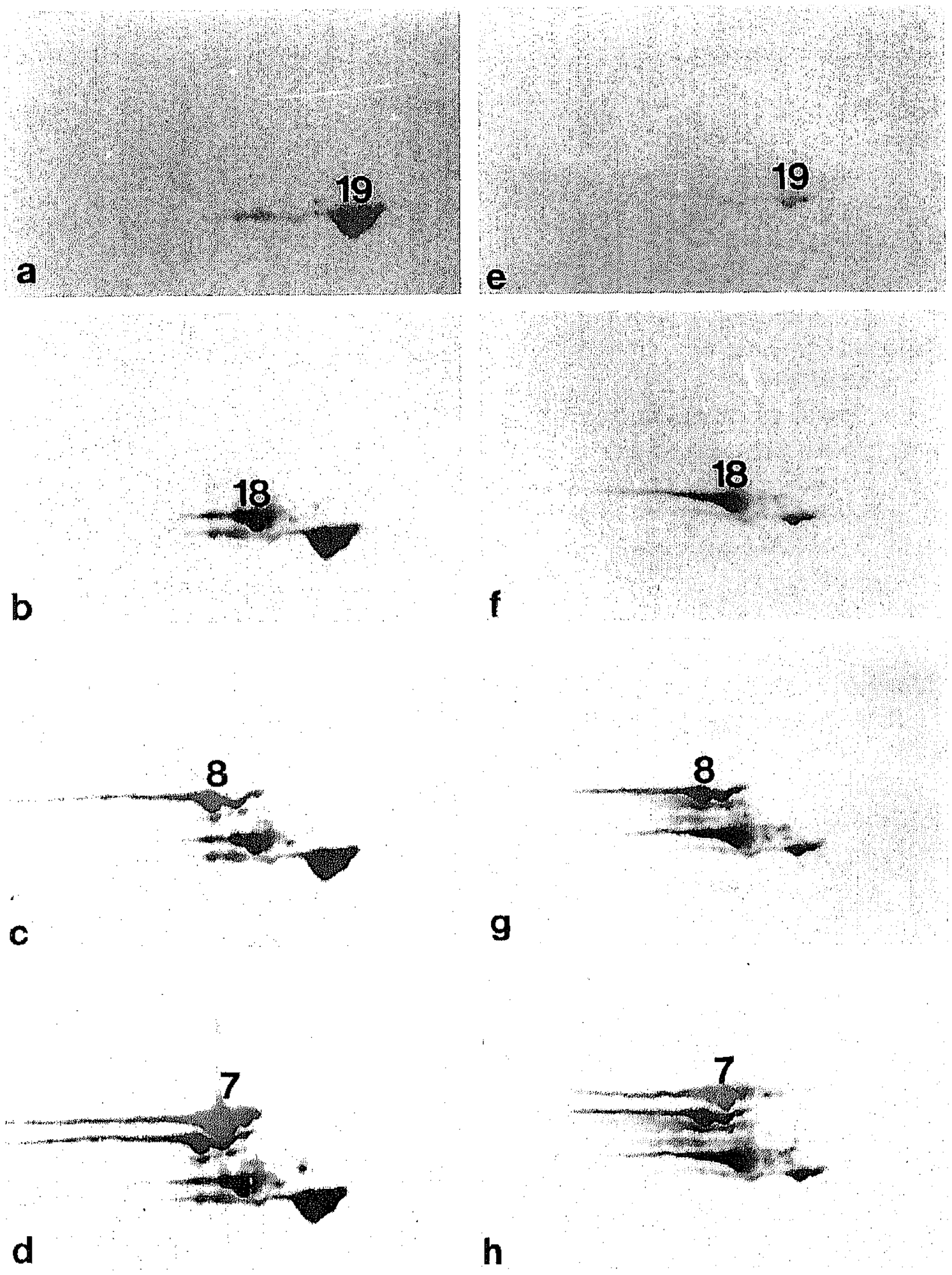
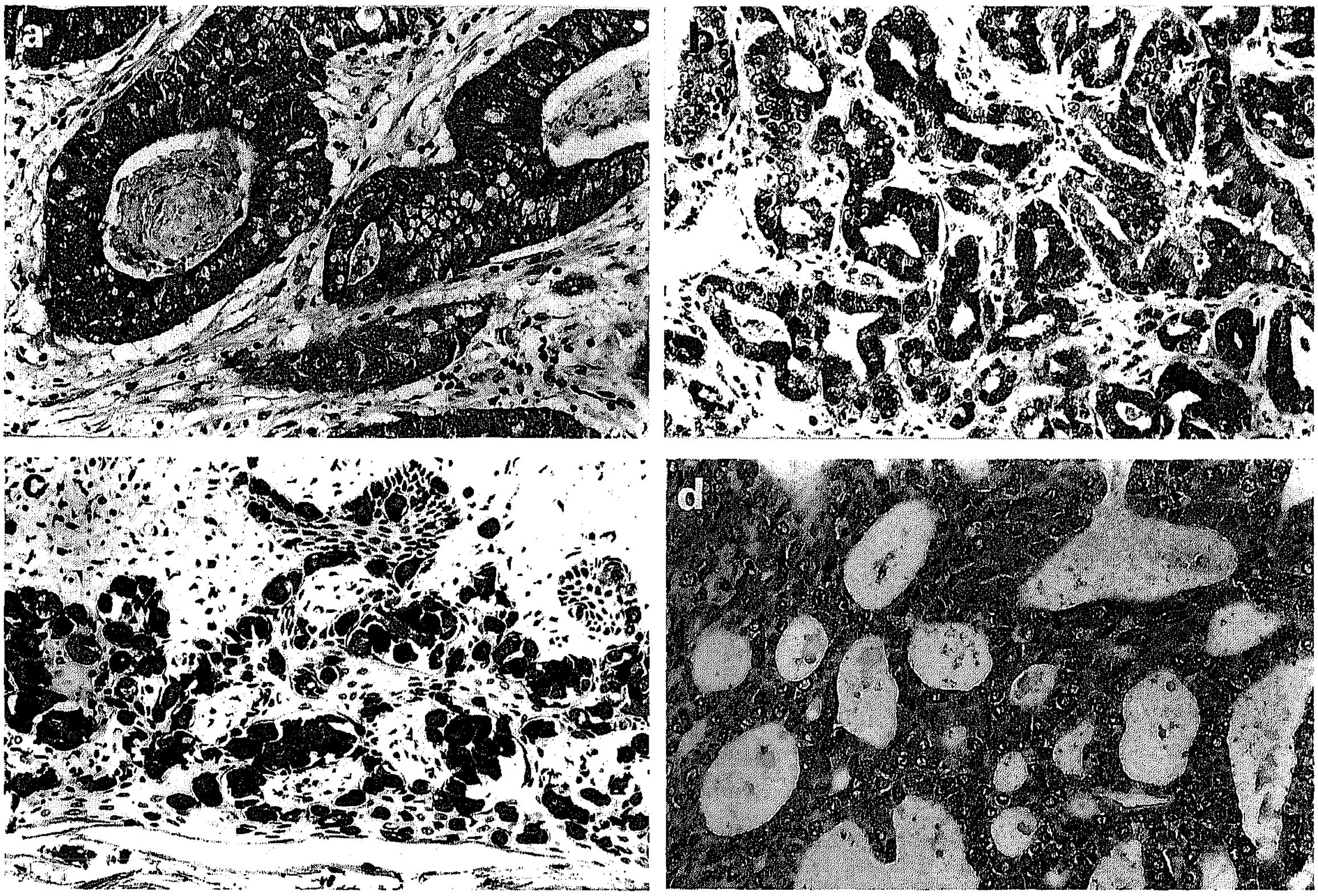


Figure 2. Two-dimensional blots of cytoskeletal preparations of RT4 cells (**a–d**) and HaCaT cells (**e–f**), subsequently (re)incubated with RCK108 (**a**, **e**), keratin 18 antibody CK18–2 (**b**, **f**), keratin 8 antibody M20 (**c**, **g**) and keratin 7 antibody RCK105 (**d**, **h**). Keratins are indicated by their respective numbers.

Discussion

When immunohistochemical tests are applied in tumour diagnosis, antibody panels invariably contain at least one keratin antibody, usually detecting a broad spectrum of keratins, such as Lu5 or a mix of AE1 and AE3, often combined with CAM5.2[11]. Since the detectability of keratins in routinely processed tissue samples varies, there is a continuous search for keratin antibodies that reproducibly recognize their epitope in formalin-fixed, paraffin-embedded tissue with a high epitope affinity. Furthermore, much effort has been directed toward enhancing accessability of antigens in such specimens[9]. The keratin antibody RCK108 is particulary promising, not only because of the wide tissue distribution of its keratin 19 antigen[6], but also because of its unusually intense and specific immunoreactivity in paraffin sections, which is limited to the epithelial cells and not seen in the surrounding mesenchymal tissues or mesenchymally derived

Figure 3. Staining reactions with the keratin 19 antibody RCK108 in epithelial malignancies. **a**, Adenocarcinoma of the stomach; **b**, adenocarcinoma of the prostate; **c**, Paget's disease; **d**, follicular carcinoma of the thyroid; **e**, medullary carcinoma of the thyroid; **f**, papillary carcinoma of the thyroid; **g**, cholangiolocellular carcinoma; **h**, hepatocellular carcinoma; **i**, small cell lung carcinoma; **j**, pituitary adenoma; **k**, transitional cell carcinoma of the urinary bladder; **l**, squamous cell carcinoma of the cervix; **m**, squamous cell carcinoma of the oesophagus; **n**, basal cell carcinoma of the skin; and **o**, astrocytoma.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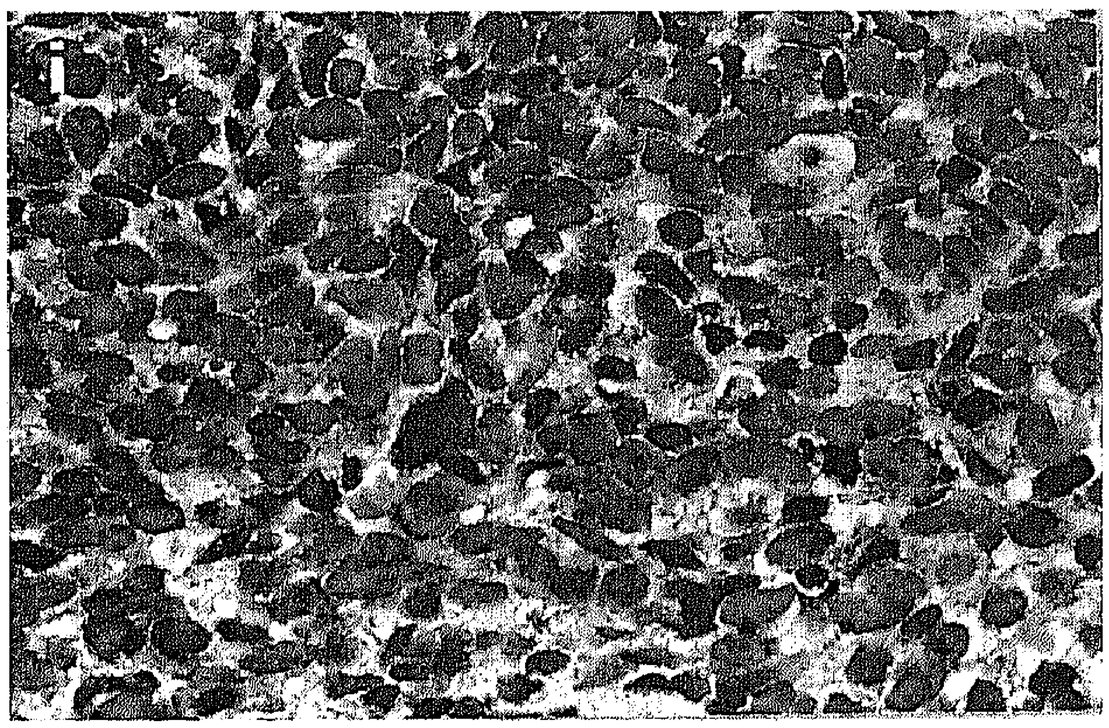

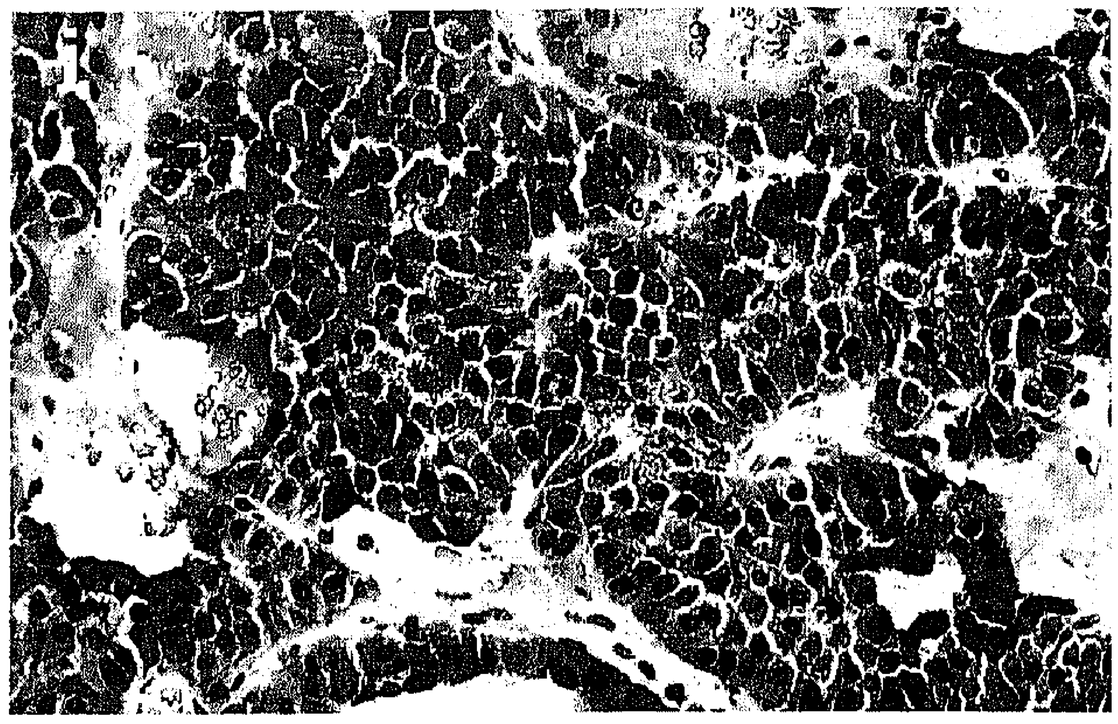

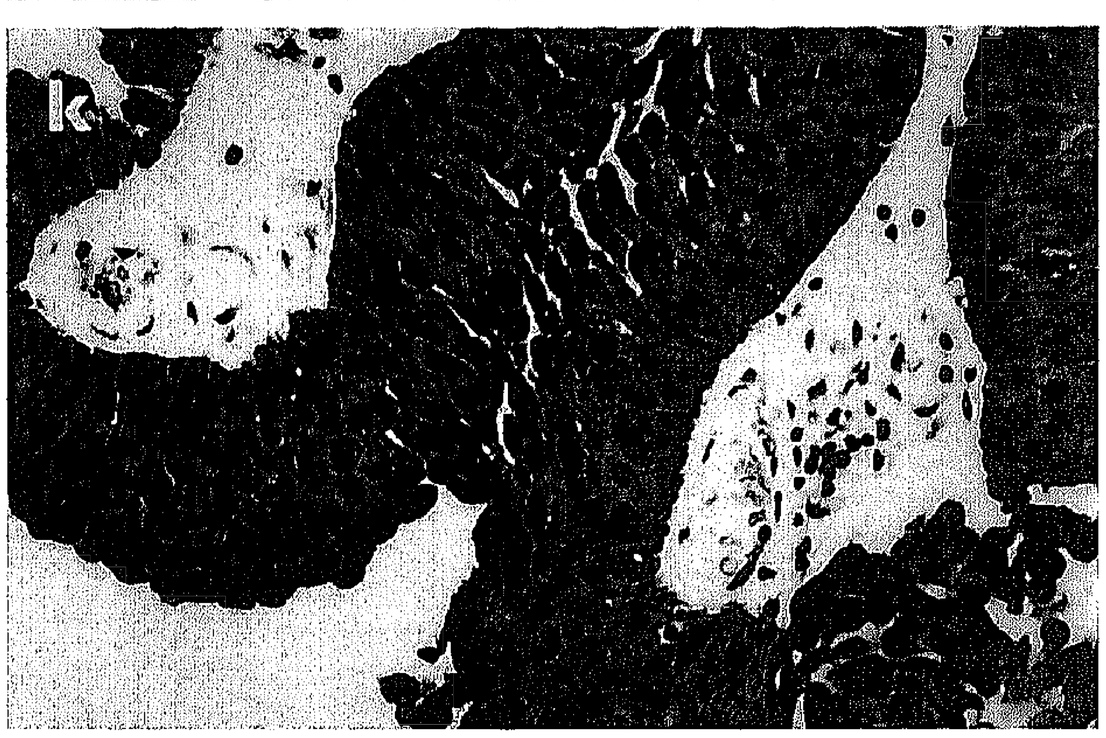

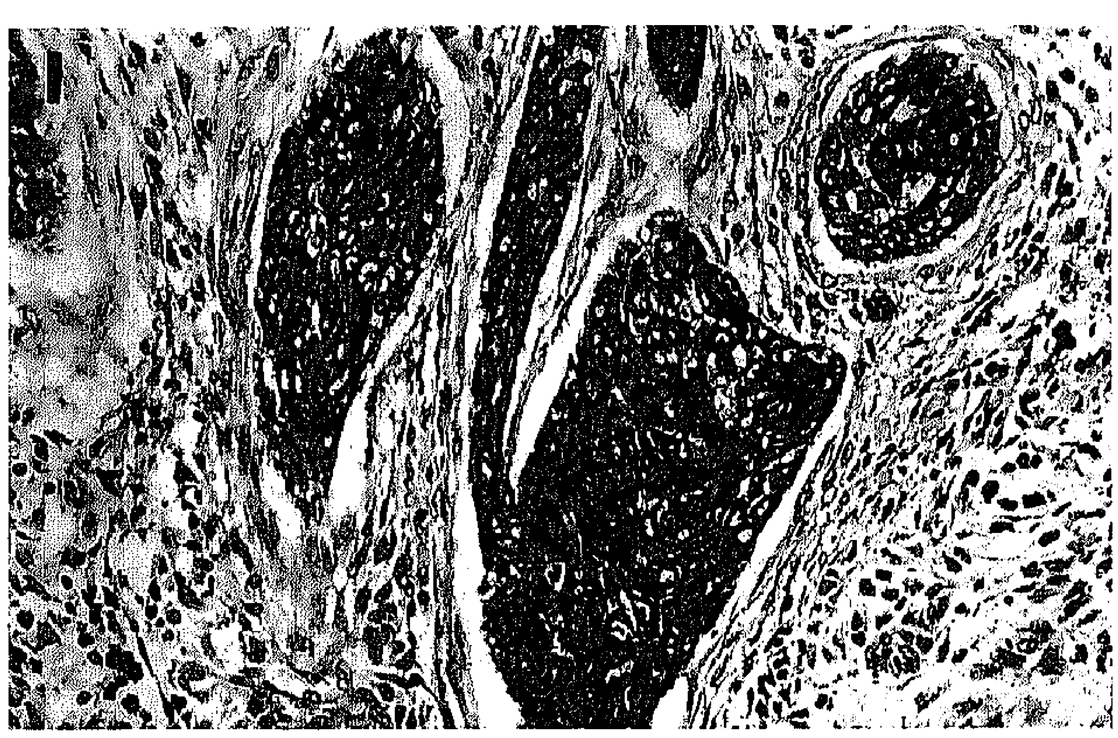

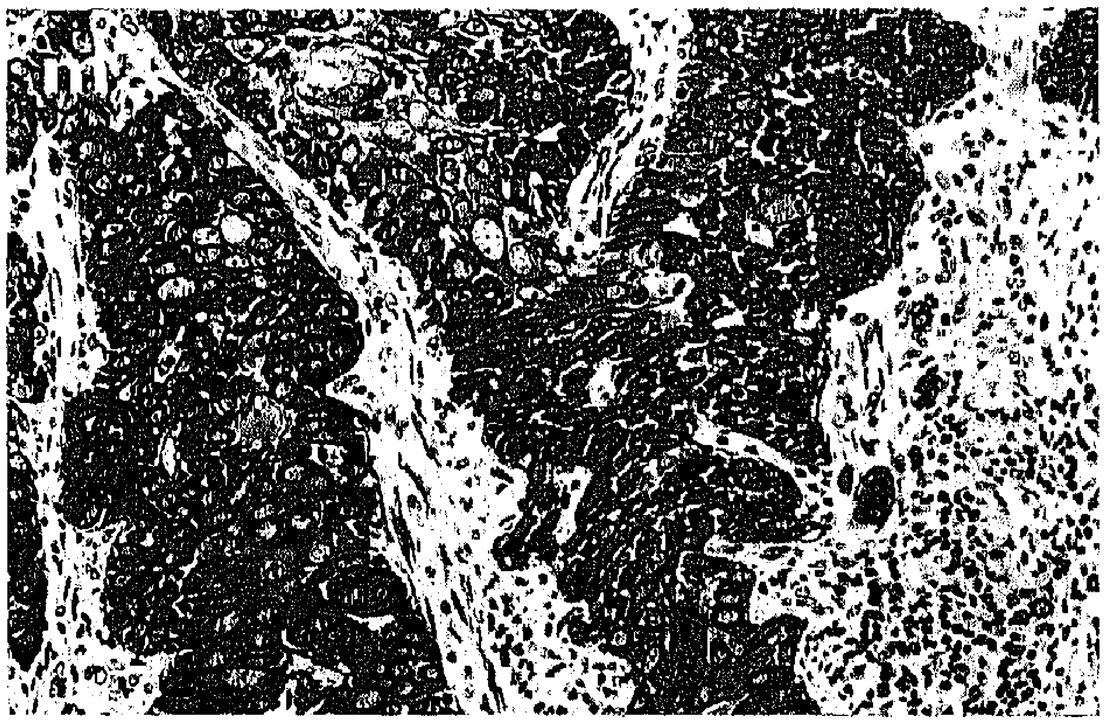

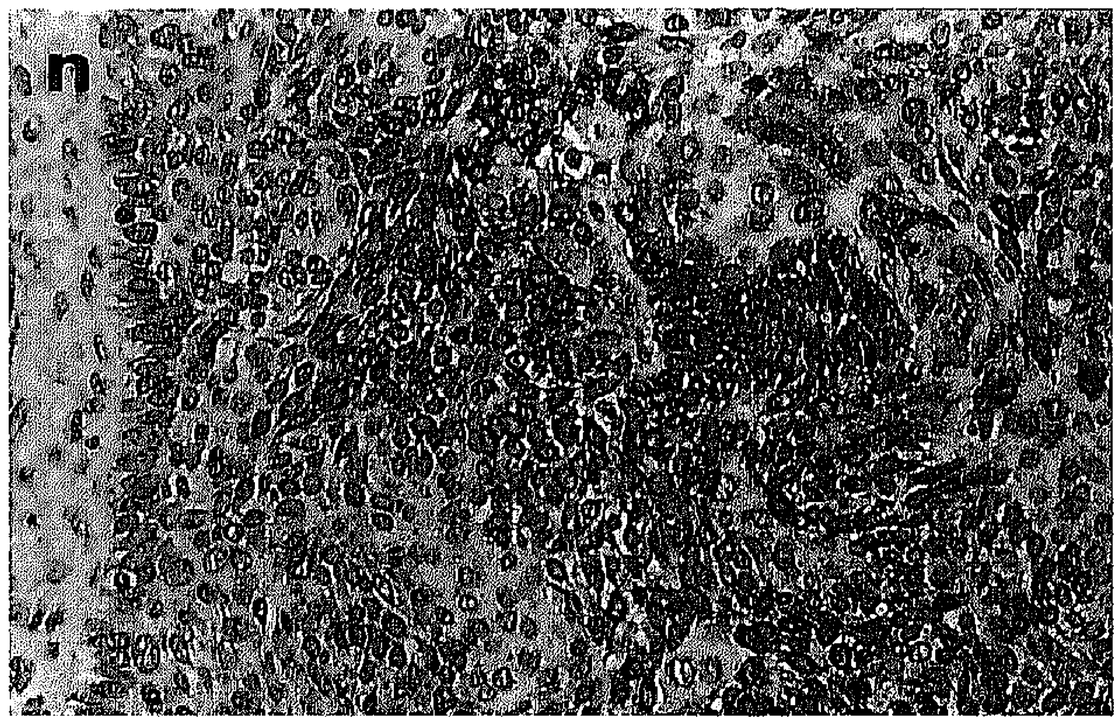

tumours (for discussion of the few exceptions, see below). The protocol to be applied for optimal immunostaining comprises a simple microwave pre-treatment step.

COMPARISON OF RCK108 REACTIVITY TO OTHER KERATIN 19 ANTIBODIES

A number of keratin 19 antibodies have been developed and applied in diagnostic pathology. Of these Ks 19–2 and RCK108 stain formalin-fixed, paraffin-embedded tissues[12–14] after protease or microwave pretreatment, respectively. Other studies investigating keratin 19 distribution[5–7,15–24] used fresh frozen tissue or methacarn-fixed tissues.

The reactivity patterns of RCK108 in paraffin-embedded normal tissues are largely comparable to the patterns observed in frozen tissue specimens tested with the other keratin 19 antibodies. However, some interesting discrepancies were noted. Born *et al.*[20] and Burns *et al.*[21], for example, noted keratin 19 staining in the myoepithelial cells of the secretory part of the parotid gland, although Burns *et al.*[21] indicated that it was difficult to discern these cells from overlying columnar cells. We could not detect keratin 19 in the myoepithelial cells of the parotid, mammary gland, salivary gland and sweat glands, which is in accord with the literature[1,22]. Basal cells of the salivary gland ducts and prostate, as well as the endocervical reserve cells showed keratin 19 expression. This may imply that bona fide myoepithelial cells can be distinguished from potentially proliferative basal cells on the basis of keratin 19 expression. Moreover, Bartek *et al.*[16,23], Taylor *et al.*[25] and Dalal *et al.*[26] noted heterogeneity in keratin 19 staining of luminal epithelial cells of the mammary gland, with absence of keratin 19 in some cells of small ducts and terminal ductal lobular units. In the underlying study, RCK108 stained all luminal cells in the mammary gland. This discrepancy may be ascribed to differences in the epitopes recognized by the various antibodies used[16,23,25,26], as well as technical aspects.

In tumours, RCK108 displayed strong reactivity in all types of epithelial malignancies, with the exception of hepatocellular carcinoma. When compared to the literature, again some discrepancies are worth noting. Schelfhout *et al.*[24] showed keratin 19 to differentiate between follicular (focal keratin 19 reaction) and papillary (strong keratin 19 reaction) thyroid carcinoma. In our study, both follicular and papillary thyroid carcinoma, and also medullary thyroid carcinoma, displayed strong immunoreactivity with the RCK108 antibody. Apparently, the high affinity of RCK108 detects low(er) concentrations of keratin 19, resulting in similar results for both types of thyroid carcinomas.

PRACTICAL APPLICATIONS

Because of its broad reactivity in normal epithelia and their malignant counterparts the RCK108 antibody is an important keratin immunoreagent to be incorporated in a basic panel of immunoreagents used for carcinoma diagnosis. In case of a positive reaction in a poorly differentiated tumour the likelihood of a carcinoma is high. In RCK108 negative cases the diagnosis of a nonepithelial malignancy should be seriously entertained. A few exceptions, in which this keratin subtype is often not detectable, include keratinizing squamous cell carcinoma and hepatocellular carcinoma. In differential diagnosis, the absence of keratin 19 in these cases opens some interesting practical applications. We found that RCK108 may distinguish between hepatocellular carcinoma (keratin 19 negative) and cholangiocellular carcinoma (keratin 19 positive), and thus be useful within this narrow differential diagnostic consideration. However, it has to be kept in mind that poorly differentiated hepatocellular carcinomas may show a positive reaction with RCK108. Van Eyken *et al.*[27,28] reported a positive keratin 19 reaction in some poorly differentiated hepatocellular carcinomas, in pseudoacinar parts of hepatocellular tumours and in hepatoblastoma. These findings are in accord with the fact that hepatocytes can transform towards a bile duct epithelium phenotype[12,27,28]. The hepatocellular carcinomas in our study were all negative for RCK108, but no poorly differentiated hepatocellular carcinomas were investigated. Another practical application for RCK108 results from its capacity to highlight the intraepithelial malignant adenocarcinoma cells in Paget's disease.

The immunoreactivity of many keratin immunoreagents with non-epithelial (tumour) cells is largely attributed to the presence of the embryonal keratin 8 and 18 subtypes[4]. This may lead to a diagnostic dilemma between poorly differentiated carcinoma and sarcoma. Application of RCK108 circumvents this problem to a large extent, although leiomyosarcomas were found to express keratin 19 in a small fraction of the cells, and astrocytomas showed focal cellular positivity for keratin 19 in all cells.

CONCLUSIONS

RCK108 antibody recognizes keratin 19 in formalin-fixed, paraffin-embedded tissue with high specificity and sensitivity. The results are highly comparable with those of other keratin 19 antibodies tested on frozen tissue

sections. Its reactivity in epithelial malignancies makes it very useful as a general carcinoma marker. It is of value in the narrow differential diagnostic considerations of hepatocellular carcinoma vs. cholangiocellular carcinoma and Paget's disease.

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