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NordiQC Assessments of Keratin 5 Immunoassays

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Abstract: This paper is number 7 in a series developed through a partnership between ISIMM and NordiQC with the purpose of reporting research assessing the performance characteristics of immunoassays in an external proficiency testing program.

Key Words: keratin 5, cytokeratin 5, immunohistochemistry, NordiQC, external quality assurance

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KEY POINTS FOR K5 IMMUNOASSAYS

- The mouse monoclonal (mm) antibody (Ab) clone XM26 is recommendable.
- The rabbit monoclonal (rm) Ab clones BSR55 and EP24 are promising.
- The rmAb clone SP27 has a superior analytical sensitivity, but unexpected reactions in lung adenocarcinomas have been observed for this clone, which can potentially cause diagnostic problems.
- The mmAb clone D5/16 B4 cannot be recommended due to an inferior analytical sensitivity and the risk of a false-positive reaction [mouse ascites Golgi (MAG)].
- For laboratory-developed assays, use an optimized protocol based on carefully calibrated Ab titer, efficient heat-induced epitope retrieval (HIER) in an alkaline buffer, and a sensitive 3-step polymer/multimer-based detection system.
- Use the pancreas, tonsil, and liver as positive and negative tissue controls.

INTRODUCTION

Keratins (Ks) are a subgroup of intermediate filaments that constitute an essential part of the cytoskeleton of all mammalian epithelial cells. In humans, there are at least 54 functional K genes, of which about half are expressed in epithelial cells, following a highly specific pattern according to the epithelial type, location, and level of differentiation. The Ks are grouped as either acidic (type I) or basic-to-neutral (type II). A pair of K molecules (one type I and one type II) forms an obligatory heterodimer, which subsequently polymerizes to form a filament.[1] The most clinically relevant type II filaments are numbered K1-8 according to decreasing molecular weight (MW) from 68 to 52.5 kDa, whereas the type I Ks are numbered K9-20, also with decreasing MW from 64 to 40 kDa.[2,3] For practical purposes, the Ks can be further grouped according to MW as either low molecular weight (LMW; K7-8 and K17-20) or high MW (HMW; K1-6 and K9-16). The LMW-Ks are mainly expressed in simple (single layered) epithelia and the superficial cells of complex (2-layered) epithelia. HMW-Ks are primarily expressed in complex, squamous, and transitional epithelia, with K5 being the most abundant and clinically relevant, usually paired with K14. In complex epithelia (eg, bronchi, breast, prostate), K5 is expressed in basal/myoepithelial cells, and in squamous and transitional epithelia, K5 is expressed in all layers, strongest in the basal and suprabasal.[1,4]

In surgical pathology, immunohistochemical (IHC) demonstration of K5 is used for multiple purposes, for example, identification of squamous cell differentiation in primary neoplasia and metastasis of unknown origin, as neoplastic epithelial cells tend to retain the K expression profile of the epithelium that they originate from.[1,4] Also, K5 is used in the distinction between usual ductal hyperplasia and atypical ductal hyperplasia of the breast because usual ductal hyperplasia contains K5 positive myoepithelial cells admixed with ductal cells, which is not the case in atypical ductal hyperplasia.[5] Both in the breast and in the prostate, invasive epithelial neoplasias can be separated from non-invasive lesions by the loss of K5-positive basal cells.[5,6]

During 2004 to 2019, the Nordic immunohistochemical Quality Control (NordiQC) program assessed IHC assays for HMW-K 7 times. Four of these assessments (runs) were based on HMW-K without further specification (2006, 2008, 2011, and 2013) and 3 were based on K5 (2004, 2016, and 2019). The aim of this paper is to present an overview of the external quality assessment results of K5 immunoassays by presenting the results from the latest NordiQC runs.

MATERIALS AND METHODS

NordiQC have accomplished 7 runs for HMW-K and K5. This paper will focus on the 2 most recent runs (46 and 55), which only included assays for K5.

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The authors declare no conflict of interest.

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A complete description of the assessment procedures is available on NordiQC's website (www.nordiqc.org) and in a review.[7] In brief, unstained slides from a tissue micro array (TMA) containing 5 to 7 cores of diagnostically relevant normal and neoplastic tissues are distributed to the participating laboratories (Labs). The Labs then stain the slides, using their routine protocol for K5, and return them to NordiQC for evaluation. In preparation of the assessment, 3 sections from different levels of each TMA are stained at the NordiQC reference Lab to monitor and validate the K5 expression levels in the material circulated. A panel of experienced pathologists and technicians assess the anonymized slides from the participating Labs, assigning a score of either "optimal," "good," "borderline," or "poor." The optimal and good stains are considered sufficient for diagnostic use. To participate in the NordiQC assessment, Labs submit comprehensive data on the protocol settings applied with detailed information on, for example, staining platform, epitope retrieval, antibody clone and format, incubation conditions, and detection system. This enables NordiQC to provide feedback and recommendations on protocol settings providing optimal results, both directly to the individual Lab in case of an insufficient result, and through publications on the website, www.nordiqc.org. The TMAs for K5 in run 46 and run 55 both comprised prostate hyperplasia, lung adenocarcinoma, and 2 lung squamous cell carcinomas. In addition, run 46 also included the esophagus, whereas the tonsil, liver, and pancreas were included in run 55. The following staining patterns were considered optimal:

- A strong and distinct cytoplasmic staining reaction of the majority of basal cells in the hyperplastic prostate glands.
- A moderate to strong and distinct, cytoplasmic staining reaction in almost all squamous epithelial cells in the tonsil.
- A moderate to strong and distinct cytoplasmic staining reaction of all squamous epithelial cells in the esophagus throughout all the cell layers.
- At least a weak to moderate cytoplasmic staining reaction of the majority of neoplastic cells in one of the lung squamous cell carcinomas in run 46, and a moderate to strong cytoplasmic staining reaction of almost all neoplastic cells in the other lung squamous cell carcinomas.
- A weak to moderate, predominantly membranous staining reaction of scattered cuboidal epithelial cells in the pancreatic intercalated ducts.
- No staining of neoplastic cells in the lung adenocarcinomas.
- No staining reaction in the liver.

RESULTS

In total, 529 K5 stained slides were submitted in run 46 (n = 266) and run 55 (n = 263). The proportions of sufficient results (scored optimal or good) were 68% in run 46 and 44% in run 55, respectively. The corresponding proportions of optimal results were 33% and 25%, respectively. In both runs, the large majority of insufficient results were due to weak or false-negative staining reactions (Fig. 1). In the remaining insufficient results, false-positive staining reactions were seen.

An overview of the performance of the most commonly used Ab clones in the 2 runs is presented in Table 1.

The number of different Ab clones used by the participating Labs was 7 in run 46 and 8 in run 55, either as concentrates (Conc) or as ready to use (RTU) products. A few of them were available as K5/K14 Ab-cocktails. In the following, each Ab clone will be described separately, except those that were only represented in run 46. The results for the RTU products based on the same Ab clone are pooled.

In both runs, the majority of assays were based on the mmAb clone D5/16 B4, accounting for 66% in run 46 and 61% in run 55, respectively. In run 46, 63% (55/88) of assays based on Conc from several vendors were sufficient, whereas only 25% (14/55) were sufficient in run 55. Similar results were seen for assays based on RTU products. In run 46, 57% (50/87) of the assays were sufficient, but only 21% (22/105) of the assays were sufficient in run 55. None of the Labs that obtained optimal results with an RTU product in run 55 used the protocol settings provided by the vendor, but used Lab optimized protocol settings or staining platform other than that intended by the vendor. The reasons for insufficient results were low analytical sensitivity and, in some cases, a false-positive reaction [probably caused by MAG reaction, which can be seen in tissue from persons with blood type A (Fig. 1)].[8,9] Different vendors use either ascites or supernatant-based Ab production for clone D5/16 B4. Overall, the supernatant formats performed better than the ascites formats, as the MAG reaction is only seen with the latter.

Another commonly used clone was mmAb XM26, which performed rather uniformly in the 2 runs. Used as a Conc, 77% (40/52) of the Labs achieved a sufficient result in run 46, and 77% (41/53) were sufficient in run 55. Three of 3 protocols based on XM26 RTU products in run 46 were sufficient. In run 55, 89% (8/9) were sufficient.

In both runs, a cocktail of the mmAb clones XM26 and LL002 (against K14) was used by a few Labs. The results were not better than for XM26 used alone.

The rmAb clone EP1601Y performed well in run 46, when used as a Conc. All 9 were sufficient. This is in contrast to the performance in run 55, where only 1 of 6 was sufficient. The same negative tendency was seen for the few RTU-based protocols with this clone. Most of the insufficient results were due to a weak or false-negative reaction.

The rmAb clone SP27 showed superior performance in both runs. The Labs that used Concs (4 in total) all achieved an optimal score. All 18 assays in run 55 based on the RTU SP27 760-4935 were sufficient.

The rmAb clones BSR55 (Conc) and EP24 (Conc or RTU) were only represented in run 55, and only used by 1 and 2 Labs, respectively, all producing an optimal result.

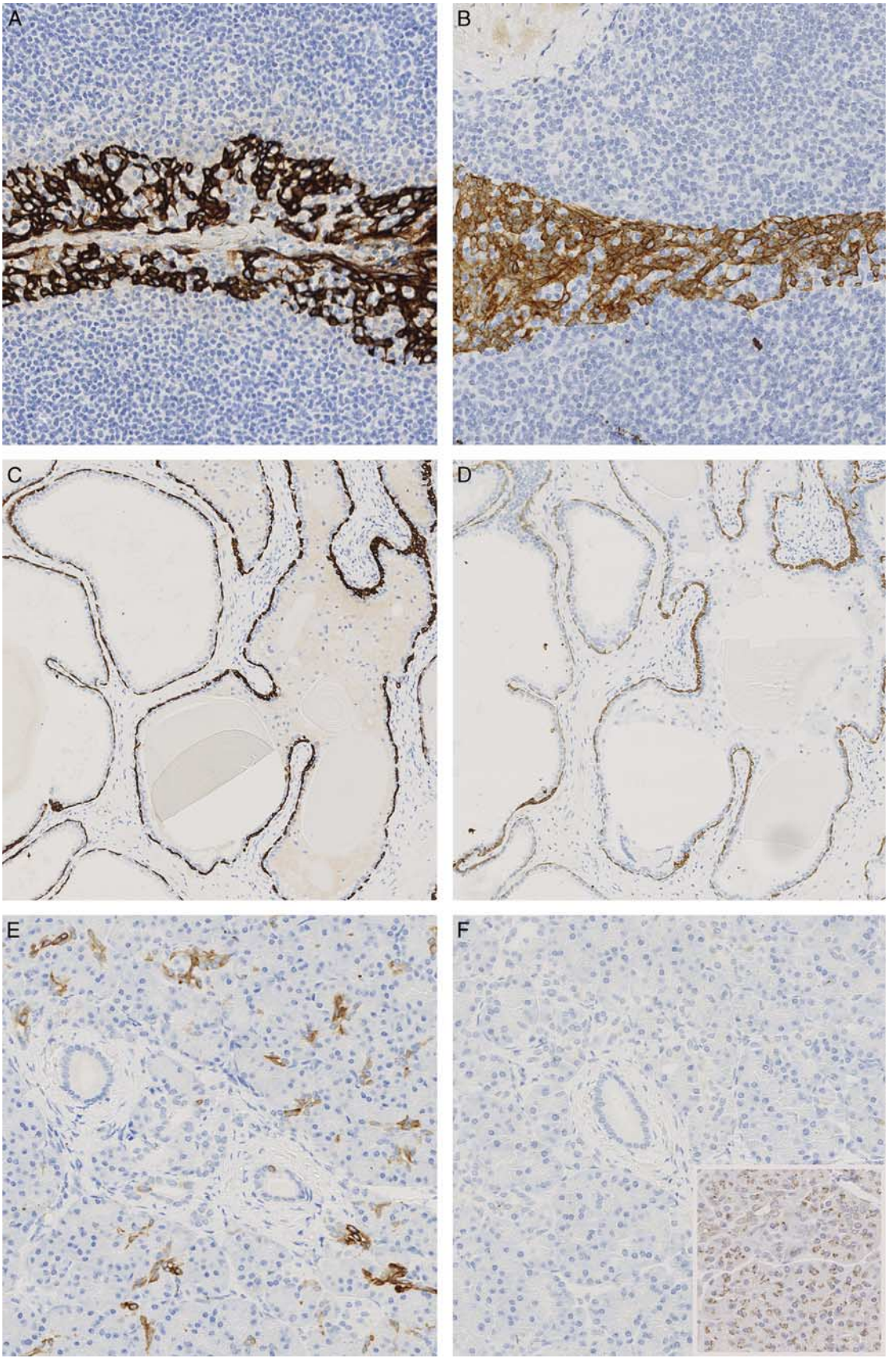

FIGURE 1. Comparison of sufficient (left column) and insufficient K5 immunohistochemical assays (right column). A, Tonsil with optimal K5 staining result with a protocol based on mouse monoclonal antibody clone XM26. There is a strong staining of almost all squamous epithelial cells. B, Tonsil with a too weak staining due to an insufficient protocol based on D5/16 B4. The reaction in the squamous epithelium is weaker than that seen in (A), but the epithelial cells are still clearly marked. C, Demonstration of basal cells in prostate hyperplasia using the same protocol as in (A). A strong and distinct reaction is seen in almost all basal cells. D, Insufficient K5 staining of prostate hyperplasia using the same protocol as in (B). There is only a weak to moderate staining reaction in the basal cells. Using this protocol for prostatic neoplastic lesions could potentially cause diagnostic difficulties. E, Demonstration of K5 in the pancreas using the same protocol as in (A). Scattered cuboidal epithelial cells of intercalated ducts show a weak to moderate predominantly membranous staining reaction. F, False-negative reaction in the pancreas with the same protocol as in (B). Inset: same core as in (F) showing a false-positive perinuclear reaction (mouse ascites Golgi reaction) in acinar cells of the pancreas with a protocol based on monoclonal antibody D5/16 B4 (Dako, M7237). In addition, the staining is false negative, as there is no marking of the intercalated ducts. All photos are from slides scanned at 200×.

DISCUSSION

Most insufficient results in both runs 46 and 55 were characterized by a too weak or even false-negative reaction in cells that were expected to be positive. In general, protocols with optimal results were based on efficient HIER (preferable in an alkaline buffer), a well-calibrated primary Ab concentration, and a sensitive detection system (preferably a 3-step polymer/multimer-based system).

The proportion of sufficient results was critically low (44%) in run 55 (2019), and much lower compared with run 46 (2016). Pancreatic tissue was included in the TMA for run 55 only. The pancreas is recommended by the International Ad Hoc Expert Committee as a low expressing on-slide external tissue control, with an expected weak to moderate, predominantly membranous staining reaction of scattered epithelial cells in the intercalated ducts.[10] This places high demands on the K5 IHC assays, and is probably one of the main reasons for the high proportion of insufficient results in run 55. Other recommendable controls are the tonsil, which should show a moderate to strong and distinct, cytoplasmic reaction for K5 in almost all squamous epithelial cells, and the liver, which should be negative.

The majority of Labs that participated in NordiQC assessments of K5 used assays based on the mmAb clone D5/16 B4. This clone has an inferior analytical sensitivity compared with the other established clones, such as XM26 and SP27. In addition to a low analytical sensitivity with a risk of false-negative results, examples with false-positive reactions were also seen, probably due to the MAG reaction. Although a few optimal results were seen in run 55, clone D5/16 B4 is challenging, and the chance of obtaining a reliable result is better with one of the more robust alternatives, which are available for all the major stainer platforms.

A high proportion of optimal results was obtained by assays based on the mmAb clone XM26 and the rmAb clones BSR55, EP24, and SP27. However, the data on BSR55 and EP24 are limited. In a recent study by NordiQC, SP27 produced a distinct positive reaction in 23% (14/62) of lung adenocarcinomas that could not be demonstrated by other K5 Abs or p40 (unpublished data). In another Lab, unexpected positive reaction by SP27 was observed in 1 of 9 lung adenocarcinomas and 1 of 9 colorectal adenocarcinomas that were both XM26 negative (unpublished data). This phenomenon can potentially cause diagnostic problems, especially if SP27 is used for the characterization of non–small cell lung cancer.

As mentioned above, the latest runs only included Ab clones against K5. In the earlier runs with HMW-K without further specification, the mmAb clone 34βE12 was commonly used, which targets a range of HMW-Ks. A large proportion of insufficient results with 34βE12 was demonstrated, often due to a false-positive reaction

TABLE 1. Staining Results for the Most Commonly Used (> 5 participants) Antibody Clones in the 2 Latest Assessments of Keratin 5 by NordiQC

	Run 46 (2016)				Run 55 (2019)			
Clone, Concentrate*	**No. Slides**	**Optimal (%)**	**Good (%)**	**Insufficient (%)**	**No. Slides**	**Optimal (%)**	**Good (%)**	**Insufficient (%)**
mmAb D5/16 B4	88	24	39	38	55	7	18	75
mmAb XM26	52	48	29	23	53	60	17	23
rmAb EP1601Y	9	67	33	0	6	0	17	83
Clone, ready to use								
mmAb D5/16 B4 IR/IS780	36	3	22	75	25	4	8	88
mmAb D5/16 B4 GA780	11	9	73	18	21	0	5	95
mmAb D5/16 B4 790-4554	38	39	39	21	56	7	25	68
rmAb SP27 760-4935	12	92	0	8	18	83	17	0
mmAb XM26 PA0468	—	—	—	—	8	50	38	12

*The results for the concentrates are pooled from several vendors.
mmAb indicates mouse monoclonal antibody; rmAb, rabbit monoclonal antibody.

(presumably cross reaction with an LMW-K).[11] It was repeatedly concluded by NordiQC that 34βE12 could not be recommended for the demonstration of HMW-K.

A more comprehensive description of the results and optimal protocol settings can be found in the assessment reports at www.nordiqc.org.[12]

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