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# Inking cell blocks improves scanner detection for diagnosis in pathology

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## Abstract

Cell blocks may be hard to be totally automatically detected by the scanner (ADS), generating incomplete whole slide images (WSIs), with areas that are not scanned, leading to possible false negative diagnosis. The aim of this study is to test if inking the cell blocks helps increasing ADS. Test 1: 15 cell blocks were sectioned, one half inked black (1HB) and the other inked green (1HG). Each of the halves was individually processed to generate a WSI stained by the H&E. 1HBs and 1HGs had similar scanning time (median 59 s vs. 65 s, $p = .126$) and file sizes (median 382 Mb vs. 381 Mb, $p = .567$). The black ink interfered less in the observation (2.2% vs. 44.4%; $p < .001$) than in the green one. Test 2: 15 cell blocks were sectioned, one half inked black (2HB) and the other left unstained/null (2HN). Each of the halves was individually processed to generate three WSIs—one HE, one periodic-acid Schiff (PAS), and one immunostained by cytokeratin AE1&AE3 (CKAE1AE3). HE and PAS WSIs from both 2HN and 2HB groups were all totally ADS and had similar scanning times and file sizes. Concerning immunostaining with CKAE1AE3: ADS (46.7% vs. 93.3%; $p = .014$), median time for scanning (57 s vs. 83 s; $p < .001$) and file size (178 Mb vs. 338 Mb; $p < .001$) were reduced significantly in the 2HN group in comparison with the 2HB. Although increasing scanning time and file size, inking the cell blocks helps increasing ADS after immunostaining, improving the safety and efficiency of the digital pathology workflow.

**KEYWORDS**
cell block, cytology, digital pathology, inking, scanning

## 1 | INTRODUCTION

Cell blocks are well-known preparations that consist in the transformation of a liquid-based cytology into a pellet-cone embedded in paraffin that can be sectioned and stained as a regular tissue fragment.[1] Producing a cell block allows the usage of complimentary techniques in liquid-based cytology samples, such as histochemistry and immunohistochemistry (IHC), among others.[2]

During the digital transformation of pathology laboratories, specific scanning protocols are to be defined for each type of preparation in order to avoid high rates of re-scan.[3] Cell block's matrix is transparent, often carrying a paucicellular content and exhibiting mixed features, between cytology and histology. These qualities may be the reason why cell blocks are frequently hard to automatically detected by the scanner (ADS), generating incomplete whole slide images (WSIs), with areas that are not scanned. The production of WSIs that lack areas that were present in the glass slide is one of the reasons to retain the WSI during the quality control check, preventing the diagnosis of false negatives by the pathologist.

  

The aim of this study is to test if inking the cell blocks helps to increase ASD, improving the safety and efficiency of the digital pathology workflow.

## 2 | MATERIALS AND METHODS

A series of 30 consecutive bronchus washes were retrieved from the liquid-based cytology containers archive of the Pathology Laboratory of Ipatimup, from December 28, 2020 to January 25, 2021. The cell content, suspended in ThinPrep® PreservCyt® solution (Hologic, Marlborough, USA) within each of the 30 ThinPrep® (Hologic, Marlborough, USA) containers, were remnants of samples that had been diagnosed as benign more than 21 days before. Each of the samples was transformed into a pellet after centrifuging at 1500 rotations per minute for 5 min, the pellet was embedded in liquefied HistoGel™ (Epredia, Massachusetts, USA) at 65°C in proportion 1:1, at 65°C. After homogenizing the pellet with HistoGel™, a short spin was performed at 2000*g* (relative centrifugal force), followed by settling at room temperature. The solidified cone was longitudinally sectioned in two equal parts as represented in Figure 1.

Tests 1 and 2 were designed and undertaken consecutively.

Test 1: 15 cell blocks were sectioned, one half inked black (1HB) and the other inked green (1HG). Each half-cone was introduced in an individual cassette and processed overnight (Donatello™ series 2 tissue processor, Diapath™, Italy) after 10% neutral buffered formalin fixation, for further paraffin embedding. One section of 3 µm from each paraffin block was obtained and was stained on the Tissue-Tek Prisma® Plus automatic stainer (Sakura™, Nagano, Japan), by the H&E technique.

Test 2: 15 cell blocks were sectioned, one half inked black (2HB) and the other left unstained/null (2HN). Each half-cone was introduced in an individual cassette and processed overnight, after 10% neutral buffered formalin fixation, for further paraffin embedding. Three consecutive sections of 3 µm from each paraffin block were obtained. The first slide was stained on the Tissue-Tek Prisma® Plus automatic stainer (Sakura™, Nagano, Japan), by the HE technique. The second slide (positively charged adhesive slide) was manually stained with PAS. The third slide (positively charged adhesive slide) after 20 min in the oven at 60°C, was submitted to perform automated IHC on the Ventana Benchmark XT™ (Ventana Medical Systems, Inc.™, Tucson, USA). The technique was performed with the OptiView DAB kit™ (Ventana Medical Systems, Inc.™, Tucson, USA) and cytokeratin AE1&AE3 (CKAE1AE3) mouse monoclonal antibody, 1:200 diluted (CellMarque™, California, USA).

Slides were automatically coverslipped on the Tissue-Tek Film® Automated Coverslipper (Sakura™, Nagano, Japan). All slides were scanned on the Pannoramic 1000® Scanner (3DHISTECH LTD., Budapest, Hungary) at 20× (0.25 µm/pixel), using the current scanning protocol validated for histological primary diagnosis in WSIs currently used in our laboratory[3] and managed by Case Center software (3DHISTECH LTD., Budapest, Hungary). The ADS of the total cone in

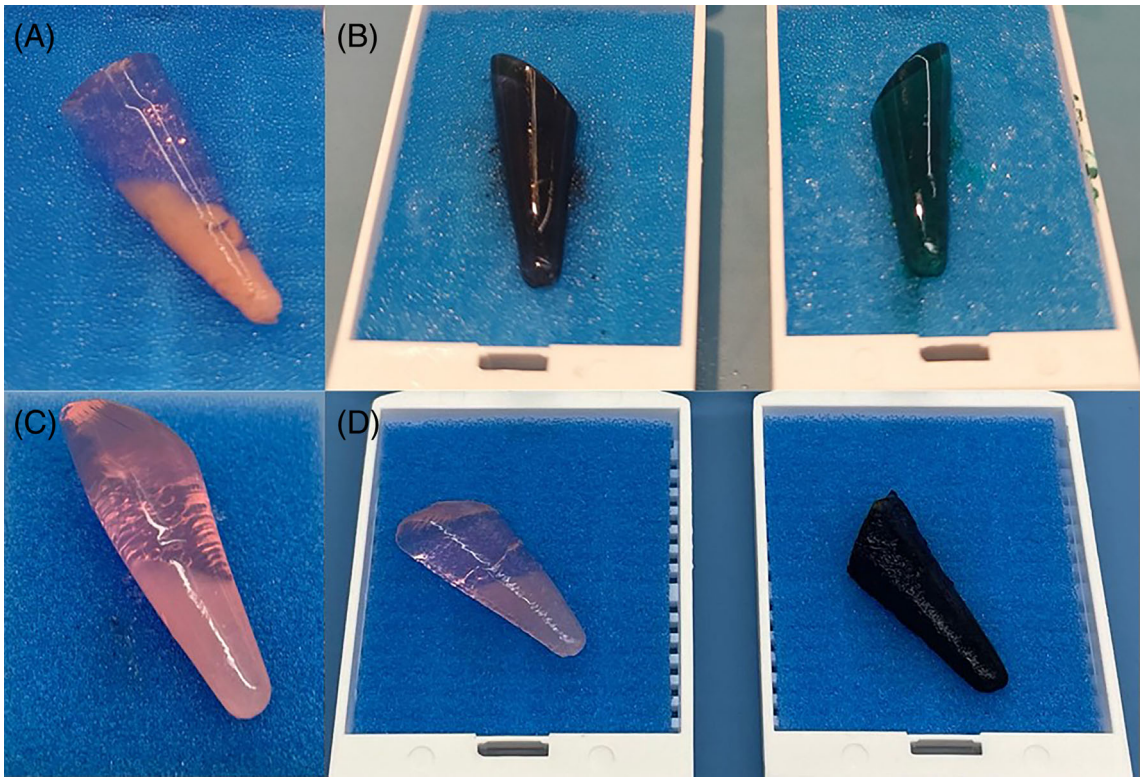


**FIGURE 1** (A) Non-inked cell block used in Test 1. (B) Cell block after sectioning in two half cones, one inked black (left) and one inked green (right). (C) Non-inked cell block used in Test 2. (D) Cell block after sectioning in two half cones, one non-inked (left) and one inked black (right). [Color figure can be viewed at wileyonlinelibrary.com]

the glass slide, the scanning time, and the size of each file produced were recorded for each WSI.

WSIs were evaluated by three pathologists, aware of the benign nature of the samples, concerning the on-focus quality (yes or no), the interference of inking (yes or no), and good diagnostic quality (yes or no), using a digital setup validated for diagnosis.[3]

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 27.0 for Windows (IBM). The Pearson's Chi-squared ($\chi^2$) test (or Fisher's exact test, if appropriate) was used for comparing qualitative variables, and the Mann–Whitney U-test (MW) was used for comparing quantitative variables. The level of significance was set at $p < .05$.

## 3 | RESULTS

### 3.1 | Test 1

During the scanning process all 15 HE slides of both 1HB and 1HG groups were totally ADS. The time for scanning HE slides of the 1HB group had a median value of 59 s (P25:47 s–P75:72 s), similar to the time for scanning the HE slides of the 1HG group that had a median value of 65 s (P25:60 s–P75:99 s) ($p = .126$; MW). The size of the files was also similar between the 1HB group (median file size 382 Mb, P25:280 Mb–P75:427 Mb) and the 1HG group (median file size 381 Mb, P25:355 Mb–P75:438 Mb) ($p = .567$; MW). The three pathologists agreed that all the 15 HE WSIs from both 1HB and 1HG groups were on-focus and were good quality. In 1 out of the 45 evaluations (2.2%) of the 1HB group and 20 out of 45 evaluations (44.4%) of the 1HG groups an interference in the observation by the ink was reported ($p < .001$; $\chi^2$). These interferences were not relevant for the diagnosis. Test 1 results are summarized in Table 1.

After these results, supporting that black ink interferes less than the green one, Test 2 was designed and undertaken.

### 3.2 | Test 2

During the scanning process all 15 HE slides of both 2HN and 2HB groups were totally ADS. The time for scanning HE slides of the 2HN group had a median value of 66 s (P25:58 s–P75:78 s), not significantly different from time for scanning the HE slides of the 2HB group that had a median value of 75 s (P25:67 s–P75:85 s) ($p = .126$; MW). The same holds true regarding the size of the files that were not significantly different between the 2HN and the 2HB groups (2HN group: median file size 340 Mb, P25:306 Mb–P75:374 Mb; 2HB group: median file size 362 Mb, P25:315 Mb–P75:444 Mb) ($p = .250$; MW). The three pathologists agreed that all the 15 HE WSIs from both 2HN and 2HB groups were on-focus and were good quality. In 5 out of the 45 evaluations (11.1%) of the 2HB group (three pathologists observing 15 WSIs) an interference in the observation by the

**TABLE 1** Results of the analysis of HE WSIs generated from cell block half-cones inked in black (1HB) and green (1HG) (Test 1).

| | ADS | | Scanning time (s) | | File size (Mb) | |
|---|---|---|---|---|---|---|
| Cell block | 1HB | 1HG | 1HB | 1HG | 1HB | 1HG |
| 1 | Yes | Yes | 48 | 60 | 279 | 365 |
| 2 | Yes | Yes | 60 | 105 | 436 | 539 |
| 3 | Yes | Yes | 59 | 75 | 392 | 438 |
| 4 | Yes | Yes | 51 | 60 | 315 | 348 |
| 5 | Yes | Yes | 40 | 61 | 280 | 353 |
| 6 | Yes | Yes | 45 | 41 | 282 | 226 |
| 7 | Yes | Yes | 47 | 65 | 276 | 364 |
| 8 | Yes | Yes | 111 | 101 | 444 | 405 |
| 9 | Yes | Yes | 59 | 65 | 376 | 381 |
| 10 | Yes | Yes | 80 | 100 | 545 | 630 |
| 11 | Yes | Yes | 72 | 61 | 427 | 398 |
| 12 | Yes | Yes | 54 | 53 | 382 | 377 |
| 13 | Yes | Yes | 72 | 91 | 398 | 475 |
| 14 | Yes | Yes | 45 | 45 | 269 | 266 |
| 15 | Yes | Yes | 91 | 99 | 409 | 438 |
| Descriptive statistics | 100% with ADS | 100% with ADS | Median 59 s (P25:47 s–P75:72 s) | Median 65 s (P25:60 s–P75:99 s) | Median 382 Mb (P25:280 Mb–P75:427 Mb) | Median 381 Mb, (P25:355 Mb–P75:438 Mb) |
| *p* | na | | .126 (MW) | | .567 (MW) | |

Abbreviations: ADS, automatic detection by the scanner; Mb, megabytes; na, not applicable; P25, percentile 25; P75, percentile 75; s, seconds.

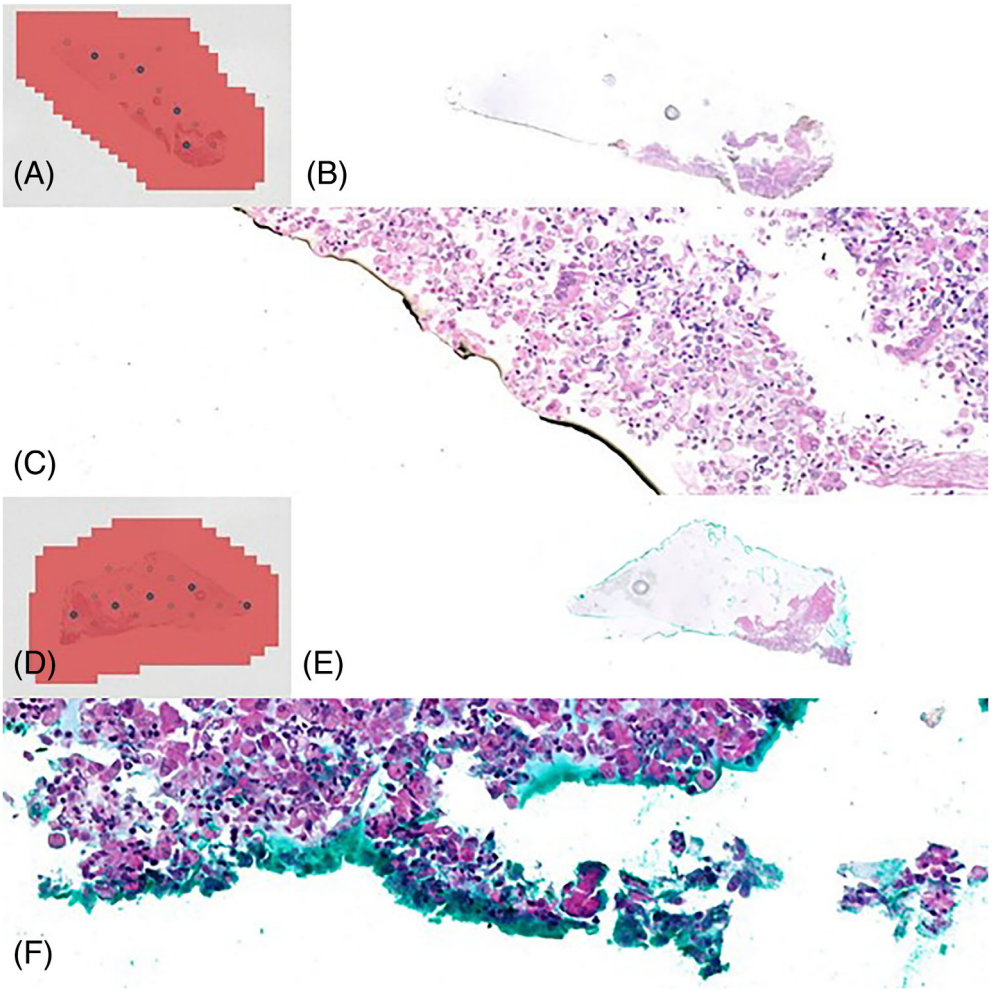


**FIGURE 2** HE WSI of inked black half cone of a cell block: (A) area of total scanner detection expressed in Case Center software, (B) HE WSI, 0.5×, and (C) HE WSI, 14.9×, demonstrating low infiltration of the ink within the cell content. HE WSI of inked green half cone of the same cell block: (D) total scanner detection expressed in Case Center software, (E) HE WSI, 0.5×, and (C) HE WSI, 20.8×, demonstrating increased infiltration of the ink within the cell content. [Color figure can be viewed at wileyonlinelibrary.com]

black ink was reported. These interferences were not relevant for the diagnosis (Figure 2).

Concerning the PAS-stained slides, all the 15 slides of both 2HN and 2HB groups were totally ADS. The time for scanning PAS slides of the 2HN group had a median value of 82 s (P25:60 s–P75:85 s), not significantly different from time for scanning the PAS slides of the 2HB group that had a median value of 85 s (P25:60 s–P75:94 s) ($p = .367$; MW). The size of the files was not significantly different between the 2HN and the 2HB groups (2HN group: median file size 355 Mb, P25:307 Mb–P75:427 Mb; 2HB group: median file size 408 Mb, P25:305 Mb–P75:468 Mb) ($p = .595$; MW). The three pathologists agreed that all the 15 PAS WSIs from both 2HN and 2HB groups were on-focus and were good quality. In 8 out of the 45 evaluations (17.8%) of the 2HB groups an interference in the observation by the black ink was reported (all eight evaluations made by the same pathologist). These interferences were not relevant for the diagnosis.

The analysis of the IHC slides stained with CKAE1AE3 is described in Table 2 and demonstrated in Figure 3. In the overall, ADS (2HB 14/15 (93.3%); 2HN 7/15 (46.7%); $p = .014$; Fisher's exact test), time for scanning (2HB 83 (80–91); 2HN 57 (49–70); $p < .001$; MW) and file size (2HB 338 (265–498); 2HN 178 (120–217); <.001; MW) are significantly reduced in 2HN group in comparison with the 2HB group. The cases that were not ADS were predominantly paucicellular ones.

The three pathologists agreed that all the 15 IHC WSIs from both 2HN and 2HB groups were on-focus and were good quality in the represented areas. In 11 out of the 45 evaluations (24.4%) of the 2HB group an interference in the observation by the black ink was reported. These interferences were not relevant for the diagnosis.

## 4 | DISCUSSION

The result of a recent survey that involved Asian and European pathology laboratories reported that the conventional microscope is still being used in digitizing workflows, not only but also due to concerns about the quality of WSIs,[4] as reported by 44.4% of laboratories with scanners.[4] The technological progress in the industry of scanners production has dramatically increased in the last years allowing every laboratory to generate diagnostic quality WSI. However, the particularities of some preparations (that may vary among institutions), either the sample content, or the staining, are main obstacles to the success of the digital workflow. The paradigmatic example is the cytology

**TABLE 2** Result of the analysis of whole slide image representative of CKAE1AE3 staining of cell block half-cones with (2HB) and without (2HN) black inking (Test 2).

| | ADS | | Scanning time (s) | | File size (Mb) | |
|---|---|---|---|---|---|---|
| Cell block | 2HN | 2HB | 2HN | 2HB | 2HN | 2HB |
| 1 | Yes | Yes | 70 | 75 | 227 | 232 |
| 2 | No | Yes | 54 | 61 | 159 | 208 |
| 3 | No | Yes | 54 | 82 | 151 | 282 |
| 4 | No | Yes | 32 | 85 | 55 | 341 |
| 5 | No | No | 57 | 83 | 184 | 313 |
| 6 | Yes | Yes | 57 | 91 | 196 | 442 |
| 7 | Yes | Yes | 47 | 60 | 120 | 201 |
| 8 | No | Yes | 51 | 91 | 109 | 507 |
| 9 | Yes | Yes | 49 | 80 | 145 | 265 |
| 10 | Yes | Yes | 77 | 100 | 243 | 556 |
| 11 | Yes | Yes | 57 | 85 | 178 | 338 |
| 12 | No | Yes | 79 | 80 | 248 | 303 |
| 13 | No | Yes | 77 | 91 | 179 | 373 |
| 14 | Yes | Yes | 45 | 123 | 75 | 687 |
| 15 | No | Yes | 62 | 80 | 217 | 498 |
| Descriptive statistics | 46.7% with ADS | 93.3% with ADS | Median 57 s (P25:49 s–P75:70 s) | Median 83 s (P25:80 s–P75:91 s) | Median 178 Mb (P25:120 Mb–P75:217 Mb) | Median 338 Mb (P25:265 Mb–P75:498 Mb) |
| *p* | .014 (Fisher's exact test) | | <.001 (MW) | | <.001 (MW) | |

Abbreviations: ADS, automatic detection by the scanner; Mb, megabytes; P25, percentile 25; P75, percentile 75; s, seconds.

samples that require, to be on focus, the usage of not only the *X* and *Y*-axis, but also the *Z*-axis in multiple planes, dramatically increase scanning time and file size.[5]

Cell blocks have been proven to be a valuable resource to achieve a stable substrate to perform complimentary techniques[2] but also to allow the production of a good quality WSI to send in teleconsultation, replacing cytology preparations.[6] Due to their intrinsic features, cell blocks are frequently hard to be ADS. The lack of ADS determines that the operator should interfere with the scanning process to improve the capture of the image, conducting to multiple attempts to re-scan the glass slide and decreasing the efficiency. Reported re-scan rates/rates of scanning errors in highly successful workflows may be as low as 1.0%–1.19%[3,7] which is one of the reasons that warrants the rapid sample management in the laboratory.

The implementation of a digital workflow requires modifications in the usual laboratory processes.[8] In this work, we demonstrate that cell blocks submitted to immunostains are ADS only in 46.7% of cases and that this ADS can be significantly improved after inking the cell block until 93.3%, without interfering in the diagnosis. The lack of ADS is mainly due to areas with negative staining or poor contrast that are ignored by the scanner. We may anticipate that this phenomenon may happen as well with poor contrast tissues. On the other hand, inking a cell block has some costs that are reflected by an increment in time for scanning and file size. The time for scanning increases from a median of 57–83 s, a difference that is far less than is needed to re-scan a glass slide. Considering the file size, there is an increment of the median from 178 to 338 Mb, increasing 160 Mb that was needed to store this WSI. This increment is due to extra pixels that comprehend ink but also slide content that was possibly missing in the incomplete WSIs. In this work, we could also understand that ink interference may be optimized by choosing the correct color to ink the cell block. In our work, the black ink produced the best results, but other colors may eventually be tested aiming to spare time and storage resources. The increment in time for scanning and file size was not observed in HE or PAS WSIs, minimizing the impact of the costs per case. An alternative procedure to control the inappropriate ADS could be the quality control of these specific WSIs with less benefits in the efficiency of the laboratory workflow.

Limitations of this study include the sample size that is small, and that was used to demonstrate a practical issue detected in our laboratory but can certainly be expanded in future studies incorporating other types of samples also difficult to scan. Similarly, the study was not blind for diagnosis and the observers were aware of the cell types they were searching for observation. This is also a limitation of the study and should be further explored in a cohort with inked samples and diverse diagnoses so that the ultimate proof of the lack of interference by the ink is tested. Ultimately, other scanners with alternative technology and detection methods may perform differently from the one used in this study and further testing with different scanners is needed to overcome this limitation of the current study.

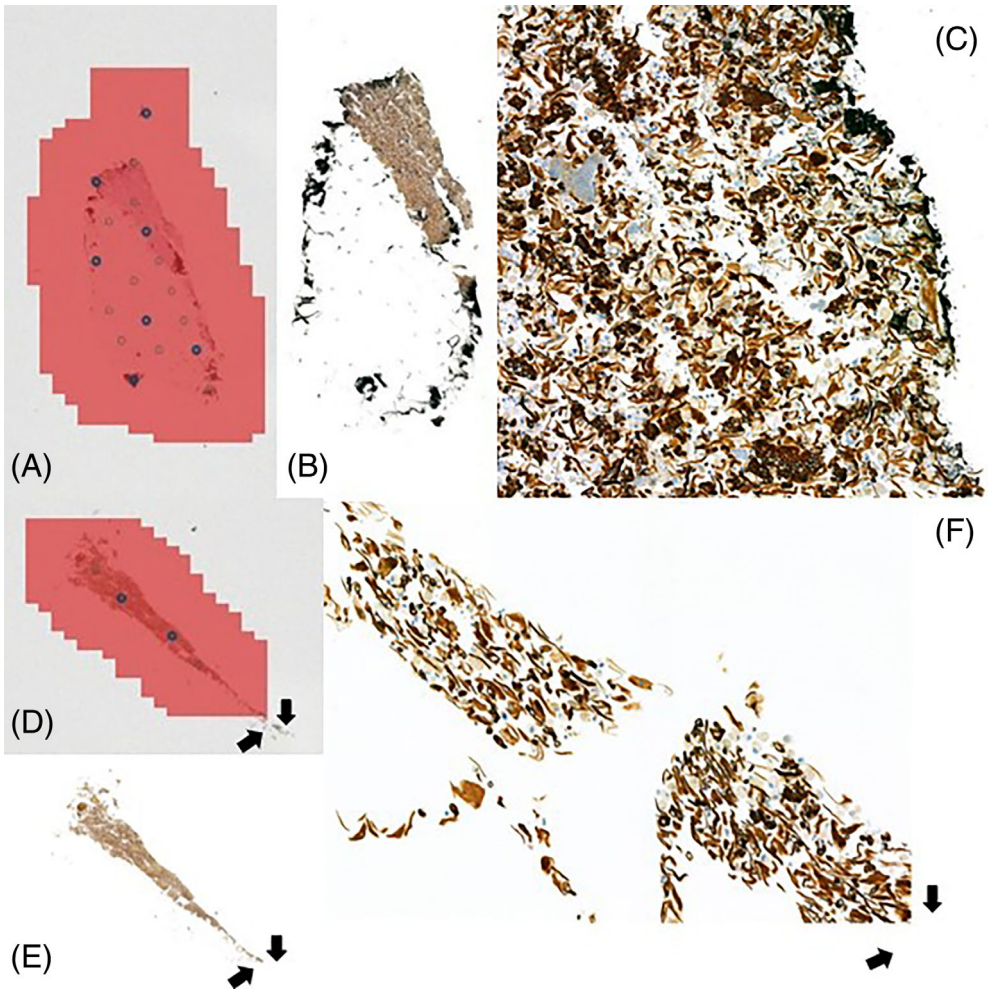


**FIGURE 3** CKAE1AE3 stained WSI of inked black half cone of a cell block: (A) area of total scanner detection expressed in Case Center software, (B) CKAE1AE3 WSI, 0.5×, and (C) CKAE1AE3 WSI, 10.0× demonstrating low infiltration of the ink within the cell content. CKAE1AE3 stained WSI of non-inked/null half cone of the same cell block: (D) partial scanner detection expressed in Case Center software with missing areas (black arrows), (E) CKAE1AE3 WSI, 0.5× with missing areas (black arrows), and (F) CKAE1AE3 WSI, 10.0× demonstrating missing areas (black arrows). [Color figure can be viewed at wileyonlinelibrary.com]

The production of incomplete WSIs after lack of ADS of cell blocks, with areas that are not scanned may give rise to false negative diagnosis. This fact justifies, per se, inking cell blocks and the implementation of a tight quality control of WSIs in every laboratory that chooses to have a digital workflow.[9]

As a conclusion, we observed that inking cell blocks improves scanner detection and allows the production of better quality WSIs for diagnosis, improving the safety and efficiency of the digital pathology workflow.

## AUTHOR CONTRIBUTIONS

Catarina Eloy, Beatriz Neves, and João Vale performed study concept and design; Catarina Eloy and Beatriz Neves performed development of methodology and writing the paper; Beatriz Neves, Mónica Curado, João Vale, Sofia Campelos, and Catarina Eloy performed observation and data collection; António Polónia and Catarina Eloy provided analysis and interpretation of data, and statistical analysis. All authors read and approved the final paper.

## CONFLICT OF INTEREST STATEMENT

Catarina Eloy consults for Mindpeak and Leica. António Polónia consults for Indica Labs. Other authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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