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Method Validation: Evaluation of the Analytical Performance of Standard Hematoxylin-Eosin Staining

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Abstract :-

➢ ***Introduction***

The optimization of diagnostic quality is a major issue in Pathological Anatomy and Cytology (ACP) to secure analytical circuits and protect the operational flow of histological analyses and molecular biology examinations. The objective of this work is to validate the analytical performance of standard hematoxylin-eosin (H.E) staining according to the evaluation criteria of slides in the laboratory.

➢ ***Materials and Methods***

Method validation involves numerous steps requiring close collaboration between pathologists and technicians. For our study, the validation covered performances such as inter-technician variability, inter-pathologist variability, and inter-block contamination. The study included 4 paraffin-embedded blocks from 2 surgical specimens, each block being sectioned by 4 different technicians on 4 different microtomes. The 16 obtained sections were stained and read by 2 expert pathologists.

➢ ***Results***

Across the 7 evaluation criteria, a preliminary concordance of 98 % was generally found, with no differences impacting the diagnosis.

➢ ***Conclusion***

The process of qualitative method validation in ACP is complex due to the multiplicity of stakeholders, the presence of several sub-processes, the difficulty in managing interfaces, and the desire to align with current regulations as a reference site (ISO 20166-4/2021 standard).4. Nevertheless, it is necessary within a quality assurance approach.

Keywords:- *Analytical Performance, Diagnostic Quality, Method Validation, Qualitative Method.*

I. INTRODUCTION

Pathological cytology anatomy (ACP) refers to the medical specialty that studies tissues, cells, and their macroscopic and microscopic abnormalities with the aim of contributing to the diagnosis of mainly oncological pathologies. The quality of the diagnosis primarily depends on the mastery of the different stages of the pre-analytical process, notably the quality of the sample, as it directly impacts the quality of the results obtained. For good performance, evaluations are necessary according to regulations. Within the framework of the normative requirements NF EN ISO 15189, ACP must verify that the adopted methods are used within their scope of application to limit the risk of errors, that they are controlled and meet the needs of patients/prescribers. The objective of this work is to validate the analytical performance of the qualitative method of standard hematoxylin-eosin (HE) staining at the Central Laboratory of Pathological Anatomy.1,3

II. MATERIALS AND METHODS

This is a prospective on-site study conducted over a six-month period at the central ACP service of the Ibn Rochd University Hospital in Casablanca. The method validation for the entire hematoxylin-eosin (HE) staining process was carried out at the local service site. The analytical performances of the staining were studied on four paraffin-embedded inclusion blocks from two different surgical specimens. Histological sections were made for each block by four different technicians using four distinct microtomes. Thus, four slides were prepared, stained with HE, and transported by each technician. In total, sixteen slides were read by two expert pathologists. The analytical performances studied in the method validation process, according to the consensus of AFAQAP and COFRAC, include: inter-technician variability, inter-pathologist variability, comparison of manual and automated methods, contamination, robustness, and reagent reliability. The evaluation results of the stains performed by each technician were recorded on anonymized forms and distributed to each pathologist for the collection of observations concerning the analysis of technical results and reading.5,6

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III. RESULTS

➢ *Inter-Technician Variability*

Regarding inter-technician variability, 448 actions were evaluated and validated by the pathologists. Sixteen slides were prepared by each of the four technicians, resulting in a total of 64 slides read, with an analysis of 7 criteria. The results of this variability are reported in **Table 1**. For the 7 evaluated criteria, a validity of 100% was obtained for all the slides studied from the four operating technicians.

Table 1: Results of the Inter-Technician Variability Study

	Sectioning	***Thickness***	***Folds***	***Tears***	***Cell Type Staining***	***Homogeneity***	***Bubbles***
Technician 1 (16 Slides)	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**
Technician 2 (16 Slides)	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**
Technician 3 (16 Slides)	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**
Technician 4 (16 Slides)	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**	**Valid**

➢ *Inter-Pathologist Variability*

Inter-pathologist variability was evaluated by comparing the analysis of the quality evaluation criteria of the slides performed by the two expert pathologists. The results showed concordance in 94% of the cases **(Table 2)**. Moreover, it should be noted that the variability observed in 6% of the cases pertains to two criteria: absence of folds and/or tears in the section, which do not impact the interpretation of the slide reading and, therefore, the anatomo-pathological diagnosis.

Table 2: Results of the Inter-Pathologist Concordance Study

	Sectioning	***Thickness***	***Folds***	***Tears***	***Cell Type Staining***	***Homogeneity***	***Bubbles***
Concordance	**100%**	**100%**	**50%**	**70%**	**100%**	**100%**	**100%**

➢ *Contamination Study*

The inter-block contamination study was conducted to assess the 4 possible levels of contamination: microtome blade, paraffin block cutting ribbon, staining reagents, and contamination between staining baths. This study revealed no contamination, indicating mastery of various levels of performance in the staining process: mastery of technical practices, personnel qualifications, adherence to hygiene rules, adherence to maintenance procedures, compliance, and initial and continuous qualification of equipment (microtome; microscope).

IV. DISCUSSION

This study involves the validation of the qualitative method for the hematoxylin-eosin (HE) staining process. HE staining is fundamental for all anatomopathological examinations at the central service of pathological cytology anatomy. Method validation is defined as **"confirmation by tangible evidence that the requirements for a specific use or intended application have been fulfilled".** 1,7. The uniqueness of this method validation lies in the multiplicity of stages—pre-analytical, analytical, and post-analytical—and the diversity of stakeholders involved in this process, necessitating close collaboration among various healthcare professionals in the central service: pathologists, laboratory technicians, quality managers, and other support staff. The objective of this validation of analytical performance is to demonstrate and verify that the method functions correctly under laboratory operating conditions and that the results provided to clinicians and patients are reliable. 2,4. Our validation study focuses on a qualitative method with extended flexible scope, "type B" 1, which is a modified method developed internally at the central ACP service of the University Hospital. Inter-operator variability was studied for the different operators involved in the HE staining process (technicians and pathologists). The results demonstrate mastery of the pre-analytical process, harmonization of technical practices, and technical staff proficiency in the ACP laboratory. The inter-block contamination study was conducted to assess the 4 possible levels of contamination: microtome blade, paraffin block cutting ribbon, staining reagents, and contamination between staining baths. This study revealed no contamination, indicating mastery of various levels of performance in the staining process: mastery of technical practices, personnel qualifications, adherence to hygiene rules, adherence to maintenance procedures, compliance, and qualification of equipment (microtome; microscope).Regarding the other analytical performance criteria of method validation, such as repeatability, reproducibility, measurement uncertainty estimation, and reference interval, these are not applicable because it is a qualitative method. Although tissue calcification may interfere with paraffin block cutting, this interference parameter could not be evaluated due to the precious nature of the samples.2,3,6

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V. CONCLUSION

Pathological Anatomy being a transversal and central discipline in patient care pathways, evaluating practices through method validation provides evidence of competence and technical reliability of anatomopathological examinations, aiming towards potential accreditation. The results of this preliminary work on the validation of the standard staining process have demonstrated its validity, robustness, and reliability within the central anatomo-pathology service.

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Annex 1: STUDY EXPLOITATION FORM

Evaluation criteria	Sectioning	Thickness	Cellular staining of different types	Uniform staining	No folds on the section	No tears in the section	No presence of bubbles between film and section	Commentary Conclusion - Validation
Slide 1								
Slide 2								
Slide 3								
Slide 4								
Slide 5								
Slide 6								
Slide 7								
Slide 8								
Slide 9								
Slide 10								
Slide 11								
Slide 12								
Slide 13								
Slide 14								
Slide 15								
Slide 16								
Summary of evaluation by criterion / Pathologist								