
**Molecular in vitro diagnostic
examinations — Specifications
for preexamination processes for
formalin-fixed and paraffin-embedded
(FFPE) tissue —**

**Part 4:
In situ detection techniques**

*Analyses de diagnostic moléculaire in vitro — Spécifications relatives
aux processus préanalytiques pour les tissus fixés au formol et inclus
en paraffine (FFPE) —*

Partie 4: Techniques de détection in situ

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Foreword

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Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Molecular in vitro diagnostics, including molecular pathology, has enabled significant progress in medicine. Further progress is expected by new technologies analyzing tissue morphology and biomolecules, such as (e.g. proteins, DNA, RNA and/or metabolites (e.g. glucose) in human tissues and body fluids.

In pathology, the majority of diagnoses are based on in situ staining of formalin-fixed and paraffin-embedded (FFPE) tissue sections. In the context of personalized medicine, classical histological staining (e.g. hematoxylin and eosin) for morphological evaluation is increasingly complemented by additional in situ detection techniques, such as immunohistochemistry or in situ hybridization, as well as molecular analysis of isolated biomolecules. For example, many regulatory bodies approved companion diagnostics in oncology are based on in situ detection techniques applied on FFPE tissue sections. Developments in personalized medicine and new technologies, such as multi-label immunostaining and computer-based analysis of digital images (e.g. generated by using a slide scanner) pose new requirements on standardization of pre-analytical procedures to obtain reproducible qualitative and quantitative results.

Profiles and/or integrity of biomolecules and their in situ localization, amount and accessibility for in situ detection in tissues can change drastically during the pre-examination process comprising specimen collection, tissue processing, embedding, transport, storage, sectioning and pretreatment for in situ detection. This makes the outcome from in situ detection in diagnostics or research unreliable or even impossible because the subsequent examination will not represent the in vivo state of molecules, but instead, an artificial profile or morphology generated during the pre-examination process.

Therefore, a standardization of the entire pre-examination process of FFPE tissue specimens intended for in situ examinations of morphology and biomolecules on FFPE tissue sections by using different in situ detection techniques, is needed.

There is multiple scientific evidence that several factors of the pre-examination phase influence the outcome (e.g. quality or quantity in terms of specificity or sensitivity) of in situ detection and, thus, can have major impact on the diagnostic results.

This document draws upon such work to organize and standardize the steps for formalin-fixed and paraffin-embedded (FFPE) tissue with regard to various in situ detection techniques in what is referred to as the pre-examination phase. This document is for the pre-examination phase of in situ detection techniques and is applicable to the whole spectrum of in situ detection techniques.

These include but are not limited to:

- Classical histological staining, e.g. Hematoxylin & Eosin staining (H&E);
- Histochemical techniques, e.g. Lipid staining, Periodic Acid Schiff (PAS) reaction, Perls' Prussian Blue reaction, Feulgen's reaction, enzyme histochemistry;
- Immunohistochemical staining (IHC) or immunofluorescence staining using antibodies (polyclonal, monoclonal or recombinant antibodies) or other affinity binders;
- Hybridization-based techniques such as RNA or DNA in situ hybridization (ISH) techniques, e.g. fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), or silver enhanced in situ hybridization (SISH);
- Molecular analysis of isolated biomolecules that can be mapped to a defined region of an FFPE section (by e.g. in situ sequencing, imaging mass spectrometry).

In this document, the following verbal forms are used:

- "shall" indicates a requirement;
- "should" indicates a recommendation;