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Review

Detection of alpha human papillomaviruses in archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens

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ABSTRACT

Formalin-fixed, paraffin-embedded (FFPE) tissue specimens stored in pathology departments worldwide are an invaluable source for diagnostic purposes when fresh clinical material is unavailable as well as for retrospective molecular and epidemiological studies, especially when dealing with rare clinical conditions for which prospective collection is not feasible. Accurate detection of HPV infection in these specimens is particularly challenging because nucleic acids are often degraded and therefore, not suitable for amplification of larger fragments of the viral genome or viral gene transcripts. This review provides a brief summary of molecular methods for detecting alpha-HPV DNA/RNA in FFPE tissue specimens. We specifically address the key procedural and environmental factors that have the greatest impact on the quality of nucleic acids extracted from FFPE tissue specimens, and describe some solutions that can be used to increase their integrity and/or amplifiability. Moreover, commonly used methods for HPV DNA/RNA detection in FFPE tissue specimens are presented and discussed, focusing on studies using polymerase chain reaction as an HPV detection method and published after 1999. Finally, we briefly summarize our 22 years of experience with HPV detection in FFPE tissue specimens.

Contents

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1. Background

Persistent infection with high-risk human papillomaviruses (HPV) from the genus *Alphapapillomavirus* (mostly HPV16) is responsible for the development of cervical and anal cancers and their immediate precursors, and a substantial proportion of vaginal, penile, vulvar, oral, and oropharyngeal cancers [1–3]. Low-risk alpha-HPV types (mostly HPV6/11) are the main etiological agents of two benign neoplasms: anogenital warts and laryngeal papillomas [4] and are rarely associated with human cancers [5–7]. A small subset of cutaneous alpha-HPV types is etiologically linked with the development of skin warts [8].

Alpha-HPVs are usually identified by the detection of their nucleic acids, predominantly DNA, mainly using polymerase chain reaction (PCR) or liquid hybridization [9,10]. In daily practice, HPV testing for the main agreed-upon clinical indications is predominantly conducted on clinician-collected cervical swab/brush specimens in various transport media or liquid-based cytology media [9,10]. When clinician-collected cervical samples are not available, and for epidemiological studies, alternative clinical specimens such as self-collected female cervicovaginal samples (brushes, swabs, lavage, tampons, etc.,), urine, oral swabs, saliva, anal swabs, penile swabs, and so on might be used for HPV testing [9–16]. Due to many reasons, fresh tissue samples are usually not available for routine HPV testing. Instead, fresh frozen tissue or tissue fixed with formalin and embedded into paraffin (FFPE) is frequently used for HPV detection after its histological assessment is finished. FFPE tissue specimens stored indefinitely in pathology departments worldwide are an invaluable source for diagnostic purposes when fresh clinical material is unavailable as well as for retrospective molecular and epidemiological studies, especially when dealing with rare clinical conditions for which prospective collection is not feasible. FFPE samples can be used for the detection of HPV up to 70 years post fixation (detailed data available in Supplementary Tables 1 and 2), given that the target molecules—nucleic acids and/or antigens—are preserved. Although in situ hybridization, as the only molecular method allowing detection and identification of HPVs in topographical relation to their pathological lesions, seems to be ideally suited for detecting alpha-HPVs in FFPE tissue specimens, the majority of researchers consider it too laborious and to have insufficient sensitivity and specificity to be used as a front-line diagnostic and/or research tool. Thus, among the available molecular methods, PCR has been the most frequently used for detecting HPV in FFPE tissue specimens [17,18].

Here, we provide a brief but critical and practical overview of molecular methods for detecting alpha-HPV DNA/RNA in FFPE tissue specimens. We specifically address the key procedural and environmental factors that have the greatest impact on the quality of nucleic acids extracted from FFPE tissue specimens, and we describe some solutions that can be used to increase their integrity and/or amplifiability. Moreover, commonly used methods for HPV DNA/RNA detection in FFPE tissue specimens are presented and discussed, focusing on studies using PCR as an HPV detection method and published after 1999. Finally, we briefly summarize our 22 years of experience with HPV detection in FFPE tissue specimens.

2. Literature search

An initial literature search was carried out on June 1, 2015 to identify the main methods that have been historically used for HPV DNA/RNA extraction from FFPE tissues. Eligible peer-reviewed studies published between 1989 and 2015 were searched for through Medline/PubMed, Web of Science, Scopus, and Google Scholar databases, using a combination of the following terms: papillomavirus, HPV, extraction, isolation, FFPE, biopsy and archival tissue specimens. A second literature search was performed on August 1, 2015 to identify the most important national and worldwide HPV-related epidemiological studies performed on FFPE tissues, investigating the etiological association of alpha-HPV with various benign and malignant mucosal and cutaneous neoplasms, using PCR protocols targeting less than 450-bp fragments of the viral genome. Eligible peer-reviewed studies published from 1999 to 2015 were selected from the databases mentioned above using a combination of the following terms: RNA, DNA, NGS, PCR, INNO-LiPA, Linear Array, SPF10, PGM09/11, GP5+/6+, papillomavirus, HPV, FFPE, archival tissue specimens, biopsy, cervix, vulva, vagina, penis, anal canal, oral cavity, oropharynx, and larynx.

3. Tissue preservation parameters affecting the yield and quality (integrity) of nucleic acids

3.1. Tissue fixation and types of fixatives

Tissue fixation is the fundamental step in processing tissue specimens for histopathological examination and long-term preservation in pathology labs worldwide. This physicochemical process stabilizes cell morphology and tissue architecture, disables proteolytic enzymes, strengthens the tissue to withstand processing and staining, and protects tissue against microbial contamination and decomposition. There are 4 major groups of chemical fixatives available for tissue preservation: aldehydes (e.g., formaldehyde, glutaraldehyde), oxidizing agents (e.g., osmium tetraoxide), alcohol-based fixatives (e.g., methyl alcohol, acetic acid), and metallic fixatives (e.g., mercuric chloride, picric acid) [19]. Formaldehyde is a small molecule existing as gas, which is usually used in the form of a 37% formaldehyde solution created by bubbling formaldehyde gas through water until saturation point. Its most common form in pathology laboratories is 4% aqueous solution, known as the 10% formalin, which can be buffered at pH 7 by acetate or phosphate. The 10% formalin is the most popular fixative due to its good preservation of morphological features in various tissues, convenience in handling, low cost, fairly fast fixation, easy processing, and wide variety of histologic techniques that can be subsequently performed [20]. Formalin stabilizes cell biomolecules by inducing crosslinks of proteins and nucleic acids via amino groups (methylene bridges), and is followed by tissue dehydration and paraffin embedding [21]. Fixation with neutral buffered formalin allows relatively good preservation of proteins and nucleic acids, and so FFPE tissues can be reliably used in translational research on DNA-, RNA-, microRNA-, and protein-based biomarkers [20,22].

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