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REVIEW

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Immunohistochemical diagnosis of human infectious diseases: a review



Hamadou Oumarou Hama^{1,2}, Gérard Aboudharam^{2,3}, Rémi Barbieri^{1,2}, Hubert Lepidi^{2,4} and Michel Drancourt^{1,2*}

Abstract

Background: Immunohistochemistry (IHC) using monoclonal and polyclonal antibodies is a useful diagnostic method for detecting pathogen antigens in fixed tissues, complementing the direct diagnosis of infectious diseases by PCR and culture on fresh tissues. It was first implemented in a seminal publication by Albert Coons in 1941.

Main body: Of 14,198 publications retrieved from the PubMed, Google, Google Scholar and Science Direct databases up to December 2021, 230 were selected for a review of IHC techniques, protocols and results. The methodological evolutions of IHC and its application to the diagnosis of infectious diseases, more specifically lice-borne diseases, sexually transmitted diseases and skin infections, were critically examined. A total of 59 different pathogens have been detected once in 22 different tissues and organs; and yet non-cultured, fastidious and intracellular pathogens accounted for the vast majority of pathogens detected by IHC. Auto-IHC, incorporating patient serum as the primary antibody, applied to diseased heart valves surgically collected from blood culture-negative endocarditis patients, detected unidentified Gram-positive cocci and microorganisms which were subsequently identified as *Coxiella burnetii*, *Bartonella quintana*, *Bartonella henselae* and *Tropheryma whipplei*. The application of IHC to ancient tissues dated between the ends of the Ptolemaic period to over 70 years ago, have also contributed to paleomicrobiology diagnoses.

Conclusion: IHC plays an important role in diagnostic of infectious diseases in tissue samples. Paleo-auto-IHC derived from auto-IHC, is under development for detecting non-identified pathogens from ancient specimens.

Keywords: Immunohistochemistry, infectious diseases, past infections, diagnosis

Introduction

Diagnosis of infectious diseases is fundamentally based on the isolation by culture of the causative pathogen and isolation by culture of pathogens remains the gold standard method for the laboratory diagnosis of infectious diseases [1]. The choice of methods used for isolation by culture depends in part on the nature of the sample, the microorganism to be identified, and the conditions under which the samples are processed and stored. However in some circumstances, rate of isolation of microorganisms from cultures in tissue biopsies may be

low and serological diagnosis can be difficult; as illustrated for an example, for the search for the tuberculosis agent *Mycobacterium tuberculosis* in biopsy specimens such as diseased lymph node biopsies [2, 3]. In recent decades immunohistochemistry has become an indispensable alternative for pathologists due to two major technical advances and the use of specific antibodies against various antigens. The application of monoclonal or polyclonal antibodies to viral, bacterial or fungal antigens in order to characterise infectious agents in immunohistochemistry is now routinely used in the diagnosis of many infectious diseases [4–6]. However, like any other diagnostic method, immunohistochemistry requires quality assurance, reproducibility and sensitivity in order to detect a targeted infectious agent. Thus, to

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avoid variations in immunostaining and to maintain the immunoreactivity of certain antigens, several factors need to be taken into account, mainly tissue fixation, tissue processing and antigen retrieval [7–9]. Furthermore, as the antigens had been recovered from ancient paraffin blocks and mummified bodies, the preservation of antigenic epitopes dating back at least a century has been demonstrated by immunohistochemical staining, despite the degradation of certain antigenic determinants in ancient tissues [6, 10]. In this study, we review immunohistochemistry techniques and protocols as applied to the diagnosis of infectious diseases, including past infections in the context of paleomicrobiology.

December 2021), and the keywords used for the search were “immunohistochemistry, immunohistochemical, human infections, diagnosis” (Fig. 1). In addition, for the Google Scholar database, “Publish or Perish” software was used to pre-select the 1,000 best articles associated with the keywords and, for the Google database, the top 10 pages (n=350) were pre-selected. Manual searches were performed for articles outside the keywords and also in the reference lists of the pre-selected articles to find other relevant sources. Experimental and animal studies were excluded from our selection and the final selection of papers was based on immunohistochemical methods and the crucial contribution that immunohistochemistry can make to the diagnosis of infectious diseases in humans.

Bibliographical methods

We searched the literature for relevant articles in the PubMed, ScienceDirect, Google Scholar and Google databases. The pre-selection of articles based on titles and abstracts was complete by July 2020 (and was updated in

The historical development of immunohistochemistry and automation

Immunohistochemical staining is derived from immunofluorescence, and dates back to 1941, when Coons and

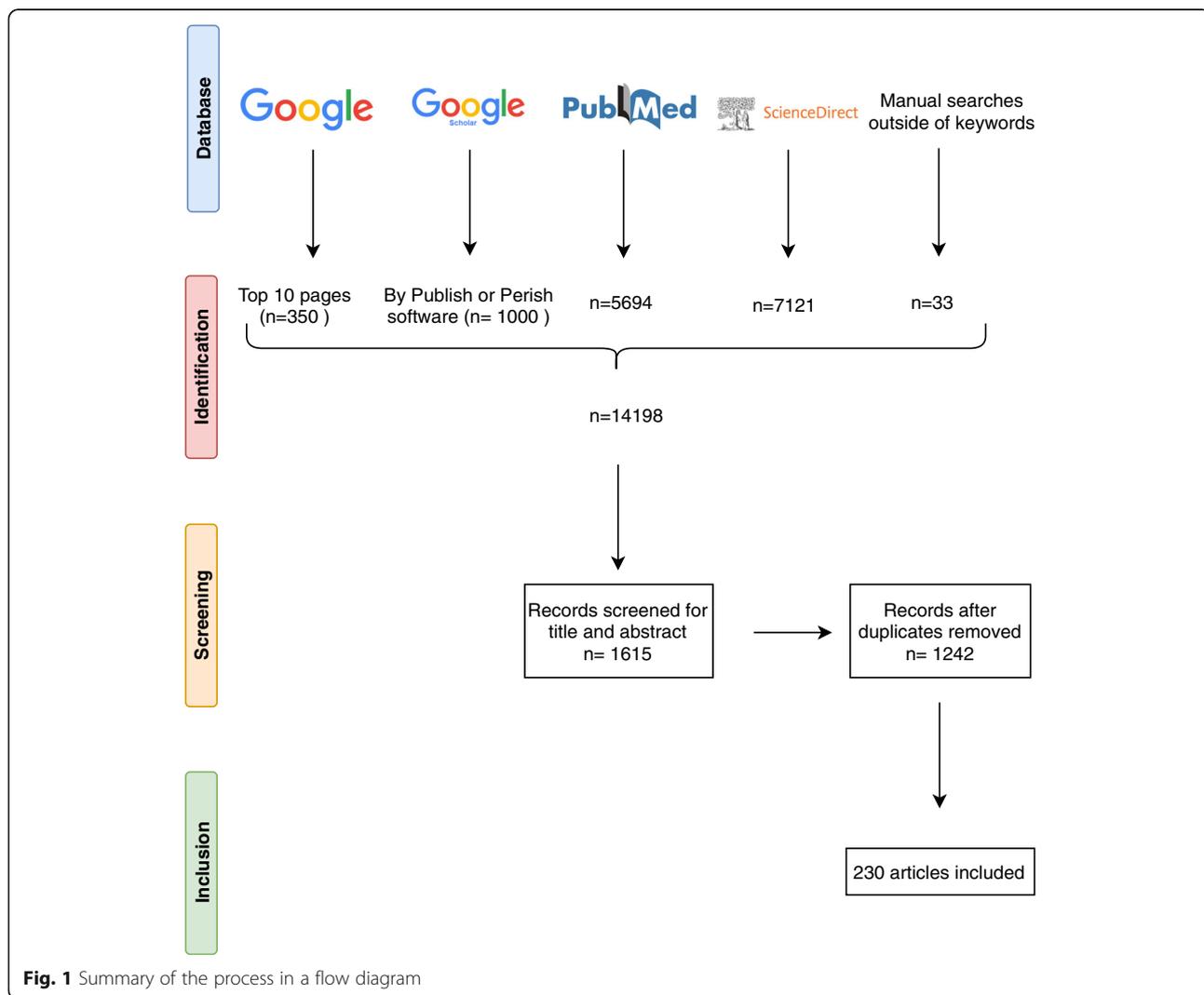


Fig. 1 Summary of the process in a flow diagram

colleagues demonstrated that tissues stained with fluorescein-conjugated antibodies became fluorescent specifically under ultraviolet light [11] (Fig. 2). Although immunofluorescence has been widely used in immunology for the diagnosis of diseases, this method has a number of limitations related to the use of fluorescein namely the natural autofluorescence of the tissues which masks the specific fluorescence, the lack of stability of the preparations, and the use of ultraviolet microscopes which are expensive and difficult to use. In order to overcome some of these shortcomings, alternative immunostaining methods have been developed. One alternative to fluorescent antibodies involves staining the tissue by methods of labelling antibodies with enzymes (Fig. 2) that react with non-fluorescent chromogenic substrates [12]. Preparations stained with antibodies and conjugated to enzymes such as peroxidase are permanent and can be observed with ordinary light microscopes, enabling the simultaneous observation of antigen localisation and tissue morphology. The use of unlabelled antibodies (peroxidase-antiperoxidase technique: **PAP**) (Fig. 2) to identify antigens by immunohistochemistry increased sensitivity [13]. A subsequent development involved using alkaline phosphatase for double immunoenzyme labelling (alkaline phosphatase and peroxidase), which was capable of detecting two antigens within the same cell [14]. However, the application of a secondary labelled antibody to biotin followed by the addition of the avidin-biotin peroxidase complex (**ABC**) (Fig. 2) proved to be much more sensitive than the unlabelled antibody method [15, 16]. In addition, the peroxidase-labelled avidin-biotin (**LAB**) method offered

even greater sensitivity than previous immunohistochemical methods [17], due to the fact that the peroxidase enzyme is directly covalently bound to the avidin molecule (Fig. 2). Epitope retrieval through enzymatic digestion was another productive step forward in the use of immunohistochemistry in formalin-fixed and paraffin-embedded tissues [18].

Due to these advances in immunohistochemistry, tissue antigen analysis became the undisputed gold standard for pathologists and anatomists for clinical diagnoses or experimental research based on tissue biopsies. The use of monoclonal antibodies made it possible to detect an increasing number use of specific markers against the antigens represented in tissues and against infectious agents from formalin-fixed paraffin blocks, and has led to increased pressure on industry. The increase in the number of tests each year in research and particularly in hospital laboratories resulted in technical problems related to the workforce, reagent stability, reproducibility, temperature variation of the different steps of an immunoassay, and a reliability crisis [19]. A viable automated immunohistochemistry system to detect tissue antigens was first invented and developed by Brigati and colleagues as an alternative to manual methods. This fully automated system (from de-paraffining to nuclear staining) overcame the restrictions on volume and discrete analysis by using the capillary action with the help of standardised software and a computer. The retrieval antigen from formalin-fixed, paraffin-embedded tissues by heat pre-treatment was a crucial step forward in the maintenance and success of immunohistochemistry [20]. This important publication resulted in the

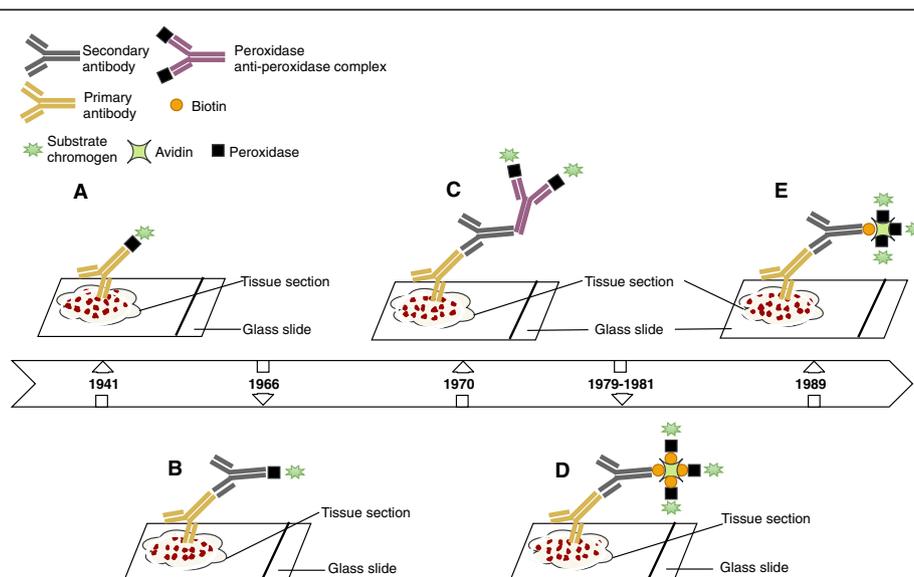


Fig. 2 Schematic representation of immunohistochemical methods. **A:** direct method, **B:** indirect method, **C:** PAP complex procedure, **D:** ABC procedure, **E:** LAB procedure

detection of antigens by immunohistochemical staining on formalin-fixed archival tissues dating back at least a century [21–23]. In the years following the automation of immunohistochemistry, considerable improvements were observed in the development of the automatons i.e., reagent cost management, reagent application, slide labelling, traceability of operations and analytical flexibility [24–26].

Immunohistochemistry procedures

The examination of hematoxylin and eosin-stained tissue sections is the first step in the diagnosis of any infectious disease from tissue samples before any other staining in histopathology [6]. The principal immunohistochemical methods are direct, indirect, PAP, alkaline phosphatase and avidin-biotin techniques [17, 27], which use antigen-antibody complexes to locate cellular antigens in paraffin sections, frozen tissues, post-mortem tissues and cell preparations.

Currently, the labelled streptavidin-biotin method is the most widely used routine laboratory method for diagnosing diseases. It has the same principle as the LAB method, i.e., the primary antibody against the antigen of interest is bound to the enzyme-labelled streptavidin (the enzyme is covalently bound to the streptavidin) through the biotin present on the secondary antibody and revealed with a chromogenic substrate [26, 28].

Tissue preparation

Immunohistochemical staining involves different steps in the technical treatment of tissues that can influence the correct interpretation of the results. These are the procedures of fixation, dehydration and paraffin embedding. Regarding fixation, two types of fixatives are commonly used, namely reticulation fixatives (e.g. formalin) and coagulation fixatives (alcohol solutions such as ethanol, methanol and acetone) [29]. Formalin is a standard fixative that has been used for decades. The increased needs of modern histopathological laboratories considered standard fixation modes too long for the preservation of immunoreactivity [30], as somewhere between 12 and 36 hours is required for complete fixation with 10% neutral buffered formalin, for example [31]. The fixation time in formalin can play an important role in obtaining a reliable result in immunohistochemistry, such as in the determination of oestrogen receptors in breast cancer, which was at least six to eight hours [32]. A large part of variations observed in immunohistochemistry were caused by the very short fixation time due to a variable mixture of cross-linking and coagulation fixation during the tissue dehydration step by ethanol [9]. In tissue diagnostics, the fixation time of formaldehyde, which is generally used at 10% in a neutral phosphate buffer, must be minimised for the effective application of the

immunohistochemical staining [33, 34]. Prolonged fixation can produce a progressive loss of certain tissue antigens used in diagnosis, such as lymphocyte antigens which are lost after three days' fixation [33]. An alternative to neutral buffered formalin and a prolonged fixation time is formalin zinc, which allows better morphological conservation and preserves immunoreactivity [30]. Coagulation fixatives are dehydrating agents, which replace water in the tissue environment and thus destabilise the hydrophobic binding of proteins. This disruption of tertiary proteins structure mainly causes the loss of functions and the insolubility of proteins [35]. This protein denaturation phenomenon does not affect all antigens and depends on the alcohol concentration, the presence of organic and inorganic substances, the pH and the fixation temperature.

However, regardless of the choice of fixation mode, fixation alone does not cause the loss of antigen recognition but rather occurs after a combination of fixation, tissue treatment and paraffin embedding. Studying the effects of different fixatives under the same conditions of treatment and embedding, found that dehydrating fixatives such as ethanol 95% or methanol 100% and alcoholic formaldehyde best preserved the immunorecognition of p53 and large spectrum keratins, while the best fixers for TGF α and p185^{erbB-2} were cross-linking fixers (unbuffered 10% formalin and unbuffered zinc formalin) [36]. After fixation, tissue treatment and embedding the tissue was dehydrated in ethanol and then the ethanol was removed by a clarification agent such as xylene, which infiltrates the tissue [37]. After the hot paraffin dissolves in the xylene, the xylene evaporates and the incorporation of paraffin into the tissue is complete once the tissue has cooled. This process is carried out using an automaton within 12 hours. Thus, using a heating mould containing molten paraffin, a paraffin block is constructed in which the tissue infiltrated by the paraffin is trapped. This paraffin infiltration into the tissue allows 4- μ m sections to be cut in order to visualise the structure, but this may also depend on the nature of the sample and the position of the tissue. The loss of reactivity of some antigens due to dehydration, paraffin embedding, paraffin wax temperature and during fixation of sections on microscopic slides has previously been discussed [29, 37]. It should be noted that the most important problem related to the variation in immunohistochemical staining is inadequate tissue dehydration. This can be avoided by renewing solutions weekly and applying consistently [9].

Antigen retrieval

Antigen retrieval by enzymatic digestion from tissue sections requires only two hours of trypsin treatment to reveal certain immunoreactive sites of the antigens [18]. However, some antigens are not revealed by trypsin digestion, with the exception of cytokeratins

and desmin [38]. This method has been replaced by microwave heating of formalin-fixed tissue sections. Subsequent methods consist in microwave-heating paraffin tissue sections at up to 100°C in the presence of metallic solutions [20]. This pre-treatment improves immunohistochemical procedures due to a considerable improvement in antigen recovery, including in long-term formalin-fixed tissues. Antigens of interest are then revealed using chromogenic substrate after several incubation steps with antibodies, as presented in the Supplementary Material.

Automation

The routine use of immunohistochemistry in diagnostic laboratories had resulted in its automation. The staining procedure remains the same as in manual immunohistochemistry, but it is controlled from a programmed computer. The advantages and disadvantages of automated immunohistochemistry vary according to the different commercially available immunohistochemistry platforms. The choice of these platforms depends on the needs of the laboratory, according to the capacity of the different systems. Unlike the automated immunohistochemical method, the manual method provides extensive staff knowledge with almost infinite flexibility in the choice of reagents and recovery methods as well as the ability to introduce certain technical variations when optimising the protocol. However, the automation of immunohistochemistry has made immunohistochemical testing reproducible, fast and accurate with processing of multiple slides at the same time, high and consistent labelling quality, analytical flexibility, standardisation of critical steps, time saving, low cost, and remote operation with a user-friendly interface and biosafety [24–26, 39]. Furthermore, despite the development of automated machines that require periodic monitoring and maintenance, the steps of tissue fixation, paraffin block construction and tissue cutting remain a human task.

Immunohistochemical detection of bacterial pathogens

Bartonella quintana (*B. quintana*)

B. quintana is a Gram-negative bacillus responsible for trench fever, bacteraemia, endocarditis, bacillary angiomatosis and chronic lymphadenopathy [40]. Trench fever was discovered during the First World War, linked to poor living conditions and lice infestation (Brouqui *et al.*, 1999; Maurin and Raoult, 1996). *B. quintana* endocarditis is a fatal infection of global importance the current approach to which involves the Duke diagnostic criteria [41]. Immunohistochemistry successfully identified *B. quintana* in the heart valves of patients with blood culture-negative infective endocarditis [42]; making histopathological

examination of heart valves mandatory for the diagnosis of infective endocarditis [43].

Yersinia pestis (*Y. pestis*)

Y. pestis is a Gram-negative bacterium responsible for plague, a zoonosis transmitted by ectoparasites including fleas and possibly lice resulting in lymph node infection (so-called bubo), which spreads as septicaemia and pneumonia [44]. Microscopy, culture and polymerase chain reaction (PCR) are the diagnostic methods used for the direct detection of *Y. pestis* [45, 46]. However, culture and direct immunofluorescence require specimens (sputum, blood or aspirations from lymph nodes) which are often difficult to obtain and dangerous to handle, hence the usefulness of immunohistochemical testing to detect *Y. pestis* in formalin-fixed tissues in order to preserve the morphological features and to minimise handling of dangerous specimens [47]. Indeed *Y. pestis* was identified intact by an immunohistochemical test inside monocytes and granular antigen staining in blood vessels (Fig. 3). Immunohistochemistry was also used to confirm pulmonary plague during a plague epidemic in Ecuador [48].

Treponema pallidum (*T. pallidum*)

Treponematoses such as yaws, bejel and syphilis are infections caused by *T. pallidum* involving the different subspecies *T. pallidum pertenue*, *T. pallidum endemicum*, and *T. pallidum*, respectively [49]. Treponemal infections, more particularly syphilis, have a worldwide impact on human health but remain indistinguishable through routine morphology and serology. Treponemes are fastidious organisms whose culture and maintenance on artificial media remains difficult, despite new techniques in microbiology [50, 51]. Silver staining of spirochetes in tissue

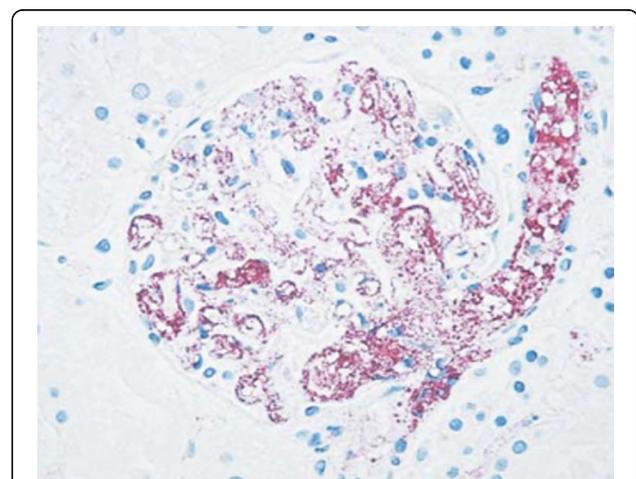


Fig. 3 Immunohistochemical stain of a kidney sample demonstrating granular antigen staining delineating the blood vessels in the glomerulus (anti fraction 1 *Y. pestis* antibody, ×100)

sections was the usual method for identifying *T. pallidum*, but the immunohistochemistry technique using a monoclonal antibody was found to be more sensitive and appropriate to avoid marked background staining and to facilitate the observation of spirochetes in secondary syphilis tissue sections [52]. Immunohistochemistry is more sensitive and specific than silver staining for detecting *T. pallidum* in tissue sections [53–55], but should be interpreted with care, as immunostaining with the *T. pallidum* antibody (Biocare) also stains some acid-fast bacilli and *Helicobacter pylori* [56]. The efficacy of immunohistochemistry for *T. pallidum* detection has been proven in biopsies of unsuspected oral lesions [57] and in biopsies of skin lesions [58], and its utility has been proven in the diagnosis of syphilitic chancre [59, 60] papulonodular secondary syphilis [61], malignant syphilis [62] and erythema multiforme caused by *T. pallidum* [63]. The combination of immunohistochemistry and PCR has been shown to be effective for the diagnosis of secondary syphilis [64] and confirmation of syphilitic orchitis in an HIV-infected young man with a false-negative Venereal Disease Research Laboratory test and *T. pallidum* agglutination test (Fig. 4A) [65].

***Chlamydia trachomatis* (*C. trachomatis*)**

C. trachomatis, an obligate intracellular bacterium, is the most common sexually-transmitted infection in the world [66, 67]. The detection of *C. trachomatis* infection is based on PCR and serology [66]. An immunohistochemical investigation incorporating a monoclonal antibody reactive against *C. trachomatis* D/K and L2 serovars (Acris Antibodies catalogue number AM00660PU-N) detected *C. trachomatis* in skin lesions in two cases of lymphogranuloma venereum (Fig. 4B) [68]. Despite the greater sensitivity of DNA detection methods (PCR and ligase chain reaction) compared to immunohistochemistry for the detection of *C. trachomatis* from fixed biopsies, the application of immunohistochemistry remains essential for pathological diagnosis [69, 70].

Other bacteria

Several microorganisms are responsible for infective endocarditis, most commonly Gram-positive cocci (such as Staphylococci, Streptococci and Enterococci), *B. quintana* (already described above), *Bartonella henselae* (the agent of cat scratch disease) *Coxiella burnetii* (the agent of Q fever) and *Tropheryma whipplei* (the agent of Whipple's disease) [42, 71–73]. However, when it comes to the antimicrobial treatment of patients and certain fastidious or non-cultivable microorganisms, identification of these bacteria by blood culture may be difficult [74, 75]. Therefore, pathological examination of valve tissue sections remains the reference technique for the diagnosis of infective endocarditis, despite the advances in molecular techniques and serological tests [43, 76]. Accordingly, immunohistochemistry is routinely used for the detection of *Bartonella* spp., *T. whipplei* and *C. burnetii*, *S. aureus*, Group A Streptococci, and for the pathological diagnosis of infectious diseases using specific antibodies [42, 72, 73, 77–79]. A related method incorporating the patient's own serum as the primary antibody, known as auto-immunohistochemistry, successfully detected bacteria in the heart valves of blood-culture negative endocarditis patients [71].

Despite cross-reactivity between the *Mycobacterium tuberculosis* polyclonal antibody and normal eosinophil granules [80], IHC can be hugely useful in identifying cutaneous mycobacteriosis [81–84] and certain bacterial infections associated with *Clostridium* species [79], *Borrelia burgdorferi* [85], *Helicobacter pylori* [86–90], *Rickettsia rickettsii* [91–93], *Chlamydia pneumoniae* [94, 95], *Orientia tsutsugamushi* [96–99], *Neisseria meningitidis* [100], *Brachyspira* species [101], and *Burkholderia pseudomallei* [102].

Immunohistochemical detection of viral pathogens

Human Herpesviruses (HHV)

HHV are intracellular pathogens that have been classified into three subfamilies and have been divided into

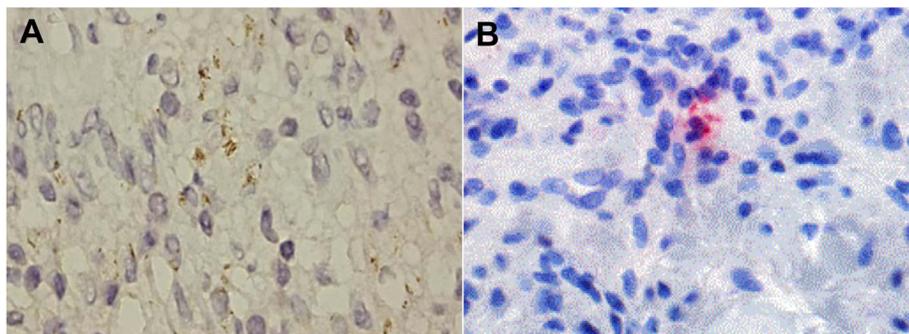


Fig. 4 A Immunohistochemistry for syphilis showing spirochetes in the cytoplasm of histiocytes (1000x, oil immersion). **B.** Immunohistochemistry showing *C. trachomatis* particles in lesional tissue

eight types based on their biological properties: *Alpha-herpesviridae* (herpes simplex virus type 1, herpes simplex virus type 2, and varicella-zoster virus) have a neurocutaneous tropism. They can replicate and spread rapidly to many cell systems causing extensive cell lysis and can establish latent infection primarily in neurosensory ganglia [103].

Herpes simplex virus (HSV) infections are among the most common diseases in humans, largely causing subclinical infections and usually manifesting in ulcerative lesions at the site of infection [104, 105]. Immunohistochemistry using polyclonal or monoclonal antibodies has been shown to be a sensitive and specific technique for diagnosing HSV infections when characteristic intranuclear inclusions or multinucleated cells are absent in biopsy specimens [2]. Discriminating HSV-1 from HSV-2 by immunohistochemistry is achieved using monoclonal antibodies and, in some cases, immunohistochemistry has been preferable to *in situ* hybridisation, as in the case of necrotic lesions, in which immunohistochemical detection has revealed the presence of HSV, while *in situ* hybridisation was negative (Fig. 5A) [106, 107].

Primary infection with VZV results in varicella and a resurgence in zoster [104]. The histological features of HSV can be observed in patients with varicella-zoster and it has been possible to identify varicella-zoster in immunohistochemistry by using a monoclonal antibody

directed against the VZV envelope glycoprotein gpl [108] and glycoprotein E [109].

Immunohistochemistry has been very useful for the diagnosis of HSV-1/2 and VZV skin infections from fixed biopsies [110–114].

Gammaherpesviridae (Epstein-Barr virus and HHV-8) are the only human herpesviruses with an established oncogenic potential, causing a variety of lymphoproliferative and neoplastic disorders [103]. The viruses of this subfamily replicate *in vitro* in lymphoblastoid cells, sometimes in epithelioid and fibroblastic cells, and are specific for T or B lymphocytes in which they can induce latent or lytic infections [103, 104].

Epstein-Barr virus (EBV) was discovered in 1964 by electron microscopy from a Burkitt's lymphoma cell line, a B-cell derived tumour. EBV is ubiquitous, infecting more than 90% of adults worldwide by establishing a lifelong persistent infection of B cells characterised by excretion of the virus in saliva, with most primary infections occurring sub-clinically in childhood [103, 104]. Immunohistochemistry identified EBV latent membrane protein 1 using tissue sections in apical and periapical periodontitis lesions (Fig. 5B) [115, 116], in patients with nasopharyngeal carcinoma [117], in immunocompetent adult [118], and in Hodgkin's disease and non-Hodgkin's lymphoma [119–121].

Human herpesvirus type 8 (HHV8), also known as Kaposi's sarcoma-associated herpes virus, is a recent

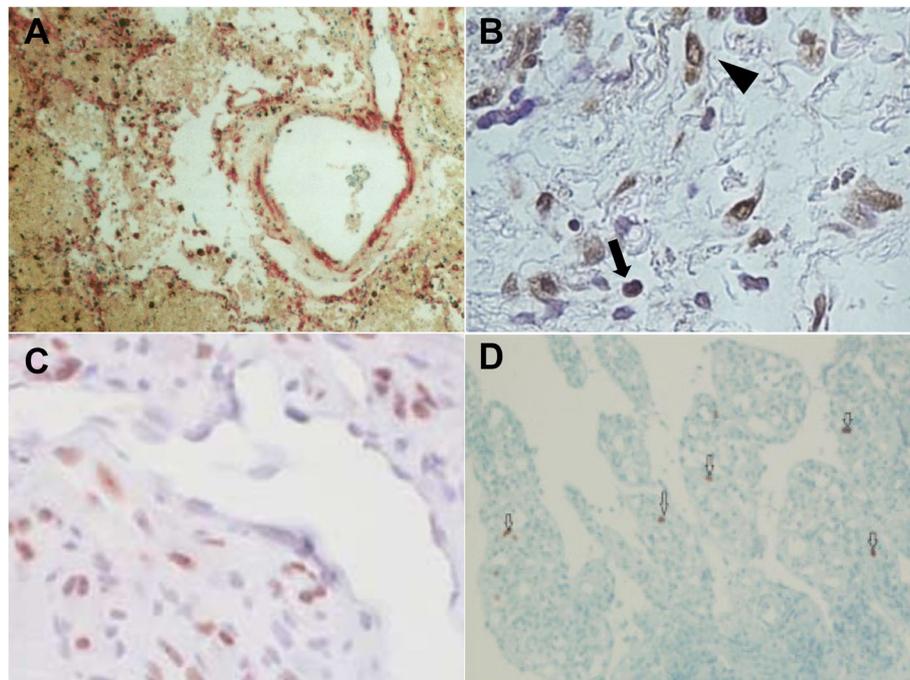


Fig. 5 **A** HSV1 immunoreactivity in the lung (IBD4 monoclonal antibody). **B** LMP-1 immunohistochemistry showing positive staining in B cells (arrows) and plasma cells (arrow heads). Scale bar = 50 μ m. **C** Immunohistochemical staining for HHV-8 LNA-1 in cutaneous patch/plaque Kaposi sarcoma. **D** Immunohistochemistry of CMV showing nuclear positive cells in ileal tissue. x20

member of the herpes family, discovered in 1994 in a Kaposi's sarcoma (KS) skin lesion in an AIDS patient, thus establishing a link between HHV-8 infection and the emergence of KS [122]. Histological diagnosis of HHV8 is problematic because of its broad morphologic spectrum and the limitation of various benign and malignant vascular neoplasms [123]. In such cases, immunohistochemistry using an anti-HHV-8 LNA-1 antibody has proven to be a reliable marker with high sensitivity and specificity for the diagnosis of KS (Fig. 5C) [124–129].

Betaherpesviridae (cytomegalovirus, human herpesvirus type 6, and human herpesvirus type 7) replicate in a limited number of cellular systems and grow slowly in cell culture. These viruses cause little cell lysis and can establish latent infections in secretory glands, the lymphoreticular system, the kidneys and certain other tissues [103].

The histological diagnosis of cytomegalovirus (CMV) in fixed tissues has proven to be the “gold standard” for the identification of viral inclusions and other cytopathic effects, despite the lack of sensitivity in some cases, such as the difficulty in interpreting atypical cytopathic features with reactive or degenerative changes [130–132]. Combined immunohistochemistry and *in situ* hybridisation tests compare favourably with culture, allowing for faster diagnosis than immunofluorescence technique or culture for early anti-CMV therapy [133]. In addition, immunohistochemistry is much more sensitive than cytomorphology and *in situ* hybridisation for the detection of CMV in smears fixed from bronchoalveolar lavage samples [134]. David and colleagues recommended automated *in situ* hybridisation or immunohistochemistry for CMV detection in formalin-fixed, paraffin-embedded tissues [135]. Immunohistochemistry using monoclonal antibodies has detected CMV antigens from fixed tissues in septic patients [136], in placental biopsies [137, 138], in the brain after liver transplantation [139], in pulmonary and gastrointestinal tissues (Fig. 5D) [140–143], in apical periodontitis lesions [116], in tumours and in peripheral blood [144], in patients with steroid refractory ulcerative colitis [145] and in one patient with ischemic colitis [146].

HHV6 was isolated and characterised for the first time in 1986 by Salahuddin and collaborators [147], from peripheral blood mononuclear cells in six patients with lymphoproliferative disorders, two of whom were co-infected with human immunodeficiency virus (HIV). HHV-6 infection usually occurs at the age of two years and is the aetiological cause of exanthema subitum, also known as roseola infantum.

Immunohistochemical analysis with a monoclonal antibody against HHV-6 envelope glycoprotein (gp60/110 kDa) in two immunocompetent individuals demonstrated the presence of numerous viral inclusions in

paracortical areas of the lymph nodes, and that atypical cells were positive for CD3 and CD4 [148, 149]. Furthermore, immunohistochemical staining has demonstrated the common presence of HHV6 in renal allografts [150], in bone marrow transplant recipients [151], in the central nervous system of neurological pathology patients and healthy people [152, 153] and in one infant infected with HIV and encephalitis [154]. HHV-6 antigens were also detected in B- and T-cell lymphoma tissues by immunohistochemistry using the polyclonal HHV6 antibody [155].

Another closely related virus, HHV-7, is also known to be the causative agent of exanthema subitum [103, 156, 157]. In 1990, HHV-7 was detected by Frenkel and colleagues in a culture of activated CD4+ T cells from a healthy individual [158].

Although rare, the detection of HHV-7 by immunohistochemistry has been successful in brain autopsy samples from unspecified encephalopathy cases [153] and in fixed normal tissues, suggesting that HHV-7 causes a persistent infection rather than a true latent one [153, 159].

Human Immunodeficiency Virus (HIV)

HIV is a retrovirus belonging to the Retroviridae family, and is responsible for Acquired Immunodeficiency Syndrome (AIDS) [103]. Biological diagnosis is based primarily on serology and PCR.

The immunohistochemical technique is rapid and efficient in identifying HIV antigens in fixed surgical and autopsy specimens [160]. Immunohistochemistry using an anti-p24 monoclonal antibody has proven useful for detecting HIV in lymph node biopsies of patients with unexplained follicular hyperplasia [161], in duodenal and rectal biopsies of AIDS patients [162], in cervical biopsy samples of HIV-infected women [163], and in a parotid gland biopsy from a rare case of HIV-associated benign lymphoepithelial cysts (Fig. 6) [164]. This approach has

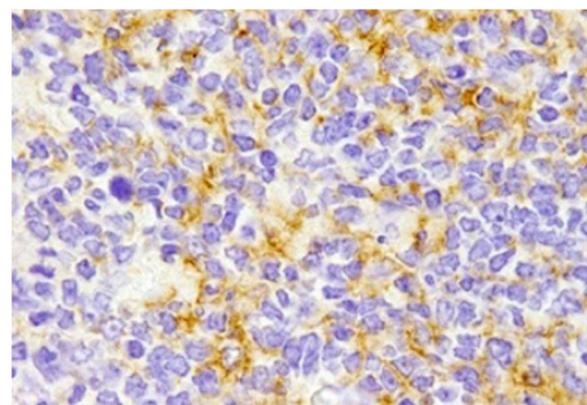


Fig. 6 Positive immunohistochemical staining for HIV-1 p24 antigen in follicular centres and dendritic cells (original magnification $\times 400$)

been recommended for the diagnosis of recent infections in which anti-p24 antibodies are not yet detectable in the serum or if morphological or clinical features suggest HIV infection [161, 165].

Other viruses

Immunohistochemistry has been of great value for the laboratory diagnosis of Ebola haemorrhagic fever, especially during the epidemic in the Democratic Republic of Congo in 1995 [166, 167], and also for the elucidation of the pathogenesis of influenza A (H1N1) and B virus infection from fixed autopsy specimens [168–170]. Immunohistochemistry has been one of the main methods used to better understand the pathogenesis of Middle East respiratory syndrome coronavirus (MERS-CoV) in humans in fixed tissues obtained from the first autopsy performed on a fatal MERS-CoV case in the world [171]. The use of immunohistochemistry in fixed tissues has been essential as a diagnostic method and for providing further evidence of enterovirus involvement in myocarditis or dilated cardiomyopathy [172–176]. Immunohistochemistry has been useful in the diagnosis and clinical management of chronic hepatitis C [177–179], in the diagnosis of yellow fever [180, 181], hepatitis E [182], rabies [183–185], severe acute respiratory syndrome (SARS) [186], in the identification and pathogenesis of SARS-CoV-2 [187–193], in the identification of infections associated with Zika virus [194], West Nile virus [195–197], adenoviruses [198–200], hantavirus [201, 202], and in the diagnosis of other cutaneous viral infections by parvoviruses, poxviruses, paramyxoviridae [203].

Immunohistochemical detection of fungal pathogens

The great majority of fungi are easily identified by hematoxylin-eosin staining alone or in combination with special stains such as Gomori methenamine silver stain and Schiff periodic acid stain which are used in routine histopathology [204, 205]. However, the morphological diagnosis of deep mycoses, ideally done by culture, can be time-consuming and often has to be made from tissue sections for smears, when cultures are not available [206]. Immunohistochemistry can be an accurate and efficient diagnostic tool for a number of important mycoses in humans and for the localisation of typical or atypical fungal elements in lesions from fixed tissue sections. Immunohistochemistry has been used for the diagnosis of pythiosis [207], fungal sinusitis [208], aspergillosis [209–211], sporotrichosis [212], and for the identification of *Candida albicans* [213], and *Cryptococcus neoformans* var. *gattii* [214].

Immunohistochemical detection of protozoal pathogens

The diagnosis of protozoal infections is difficult because of the distortion of the morphology of the parasite due to the necrosis or autolysis of tissues or in cases of unusual presentation of the disease, hence the usefulness of immunohistochemistry (Arnold et al., 1997).

Despite the higher sensitivity of PCR compared to immunohistochemistry for the diagnosis of leishmaniasis, the use of both tests together has been shown to be more effective for routine diagnosis [215, 216]. Immunohistochemistry significantly improves the diagnosis of cutaneous leishmaniasis in histological sections and allows the patient's immune response to be evaluated [217–219]. In addition, immunohistochemistry has been useful in the diagnosis of toxoplasmosis [220–222], echinococcosis [223, 224], malaria [225] and for the detection of *Trypanosoma cruzi* [226, 227].

Paleoimmunohistochemical detection of pathogens

The application of histological methods for the detection of pathogens in ancient tissues has provided information for better understanding the origin and spread of infectious diseases [6]. Currently, the representation of past epidemics relies on molecular biology, which may be an approach limited to pathogens in the event that the altered DNA prevents the amplification of pathogen-specific DNA sequences. Immunohistochemistry has been successfully used in a number of paleopathological studies, including the detection of bacteria such as *T. whipplei* and *R. rickettsii* in paraffin blocks at least a century old from autopsy cases [22, 23], the sporadic typhus agent *Rickettsia typhi* in paraffin-embedded tissue blocks from the Second World War (Hamburg, Germany, 1940-1944) [228], and the detection of a parasite (*Taenia solium*) from stomach tissue sections dating from the Ptolemaic period [21]. These studies demonstrated that the antigenicity of proteins could be preserved in ancient tissues. We invented microbial paleoserology, to reveal an epidemic of recurrent fever in a 16th-17th century French garrison, which had been missed by real-time PCR [229]. This demonstrates the interest of applying other complementary approaches allowing the detection of antigens or antibodies specific to the pathogens. In that pioneering work, total immunoglobulins previously demonstrated in ancient dental pulp [230], were extracted and applied to inactivated pathogens used as antigens in a so-called “mini blot” format [229]. Extending these observations to the detection of unidentified pathogens, we are now applying autoimmunohistochemistry used for the detection of pathogens in modern-day tissues, to ancient samples, mainly dental pulp. We call this new, antigen-based detection “paleo-autoimmunohistochemistry”.

Abbreviations

IHC: Immunohistochemistry; PAP: Peroxidase-antiperoxidase technique; ABC: Avidin-biotin peroxidase complex; LAB: Peroxidase-labelled avidin-biotin; TGF- α : Transforming growth factor alpha; *B. quintana*: *Bartonella quintana*; *Y. pestis*: *Yersinia pestis*; *T. pallidum*: *Treponema pallidum*; *C. trachomatis*: *Chlamydia trachomatis*; HHV: Human Herpesviruses; VZV: Varicella-zoster virus; HSV: Herpes simplex virus; EBV: Epstein-Barr virus; KS: Kaposi's sarcoma; CMV: Cytomegalovirus; HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; MERS-CoV: Middle East respiratory syndrome coronavirus; SARS: Severe acute respiratory syndrome

Supplementary Information

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Additional file 1: Supplementary Material

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Authors' contributions

H.O.H. and M.D.: performed the conceptualization and methodology of the review; H.O.H.: software; M.D., H.O.H., G.A. and H.L.: analysis and validation; H.O.H. and M.D.: writing and original draft preparation; H.L., G.A. and R.B.: participated the reviewing and editing; M. D.: Supervision. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

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