Chapter 32
Infectious Diseases

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Abstract Detection and identification of infectious microorganisms involves the use of conventional immunohistochemistry in addition to many other techniques including culture, serology, histochemistry, in situ hybridization, polymerase chain reaction and direct fluorescence antibody assays. This updated chapter takes into consideration all of these techniques while answering questions about bacterial, mycobacterial viral, fungal and protozoan testing. The best techniques and testing conditions are described for dozens of the most clinically relevant microorganisms. The role of immunohistochemistry versus alternative techniques is clearly presented. Photomicrographs present the characteristic feature of optimized staining techniques. Topics for each organism are discussed including the sensitivity and specificity of the tests, how fixation and retrieval affect the results, when protease should be considered in an assay, and how these tests could be incorporated into your clinical practice.

Keywords Bacteria • Bacillus anthracis • Bartonella henselae • Brucella melitensis • Francisella tularensis • Helicobacter pylori • Leptospirosis • Lyme disease • Rickettsia rickettsii • Rocky Mountain spotted fever • Treponema pallidum • Yersinia pestis • Pseudomonas aeruginosa • Mycobacteria • Bacille Calmette-Guerin (BCG) • Mycobacterium avium • Paratuberculosis • Mycobacterium tuberculosis • Mycobacterium bovis virus • Cytomegalovirus • Epstein-Barr virus • Hepatitis C virus • Human herpes virus type 6 • Human herpes virus type 8 • Human papillomavirus • Herpes simplex virus • Influenza A virus • Ljungan virus • Parvovirus B19 • Rabies • Small Pox • Variola • Varicella-Zoster virus • Viral hemorrhagic fevers • West Nile virus • Fungus • Protozoan • Aspergillus • Blastomyces • Coccidioides immitis • Cryptococcus neoformans • Histoplasma capsulatum • Pneumocystis carinii • Leishmaniasis

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32.1 FREQUENTLY ASKED QUESTIONS

For all of these immunostains discussed in this chapter, freshly-cut paraffin sections are paramount. At ambient temperature, antigenicity decreases significantly within a week or so. Therefore never use "vintage sections". To store freshly cut paraffin sections for future immunohistochemical staining for infectious organisms, dip them in the paraffin bath, thereby coating ("sealing") the entire glass slide and the mounted paraffin section. This will preserve antigenicity.

32.2 What Tests Are Available to Detect Bacteria?

For most bacteria, isolation in culture remains the gold standard for diagnosis. Serologic studies, in situ hybridization (ISH), polymerase chain reaction (PCR) and direct fluorescent antibody assay (DFA) are available. Although immunohistochemistry (IHC) is commonly used in veterinary medicine, it has become standard only for the detection of *Treponema pallidum* (*T. pallidum*) in humans. It is also used in some laboratories for the detection of *Rickettsia* and *Chlamydia* [1].

Bacillus anthracis

Colorimetric IHC assays using a multistep indirect immunoalkaline phosphatase method with anti-*B. anthracis* cell wall (EAII-6G6-2-3) and *anti-B. anthracis* capsule (FDF-1B9) monoclonal antibodies have been developed, but are not in widespread use [2]. PCR assays show considerable promise in this setting, and ELISA assays are also in clinical use [3, 4].

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32.3 How Sensitive Are the Tests?

During the bioterrorism scare of 2001, 117 skin biopsy samples were tested by the Infectious Disease Pathology Activity (IDPA) of the Centers for Disease Control and Prevention (CDC). Of these, 8 were positive for *B. anthracis* by IHC [5]. One advantage is that IHC assays can demonstrate bacilli, bacillary fragments, or granular bacterial fragments in formalin-fixed tissues even after 10 days of antibiotic treatment [6].

32.4 How Specific Are the Tests?

Limited data are available.

32.5 What Can Affect the Test?

The type of specimen is critical. The diagnosis of cutaneous anthrax should be made with skin biopsies from both the center and periphery of the eschar. For inhalational anthrax, pleural effusion cell blocks, pleural biopsies, and mediastinal lymph nodes demonstrate the largest number of bacilli.

Bartonella henselae

As microbiologic detection of *B. henselae* is problematic and molecular testing is not widely available, IHC assays are promising and ELISA assays have been developed [7, 8].

32.6 How Sensitive Is the Test?

A study of 24 samples of cat scratch lymphadenitis and 14 control specimens compared IHC based on a monoclonal antibody (mAB) with silver staining, polymerase chain reaction (PCR) detection and serologic testing for B henselae. Sensitivity was as follows: mAB 6 (25 %) detected, Steiner silver stain 11 (46 %), and PCR 9 (38 %). Interestingly, only 2 cases (8 %) were positive for all 3 studies [9].

32.7 How Specific Are the Tests?

Control tissue was consistently negative with both IHC and PCR, so while sensitivity is low, specificity appears high. There are now commercially available antibodies against B. henselae and B. quintana, both of them pathogens of bacillary angiomatosis. These two antibodies are not cross-reactive, therefore both immunostains should be performed. High sensitivity. Good specificity.

32.8 Does Fixation Affect the Test?

Testing can be performed on formalin-fixed, paraffin-embedded tissue, but optimal retrieval methods remain to be defined.

Brucella melitensis

B. melitensis, a widespread zoonotic pathogen, is a significant cause of abortion in farm animals and a cause of human sepsis.

32.9 How Sensitive Are the Tests?

In a study of 110 naturally occurring aborted sheep fetuses, *B. melitensis* antigens were detected by IHC in 33 of 110 fetuses (30 %). Breakdown by tissue included lung (22.7 %), liver (19 %), spleen (11.8 %), and kidney (5.4 %) [10].

32.10 How Specific Are the Tests?

Limited data are available.

Chlamydia

Identification of the organism in formalin-fixed tissues can be performed using IHC. PCR and ELISA assays are also used [11–13].

32.11 How Sensitive and Specific Are the Tests?

Limited data are available, but our own experience with IHC in the setting of lymphogranuloma venereum has been very positive and we recommend it highly.

32.12 Does Fixation Affect the Test?

Limited data are available.

Francisella tularensis

Both IHC and DFA have been used to demonstrate the bacteria in formalin-fixed tissues [14].

32.13 How Sensitive Are the Tests?

Limited data are available.

32.14 How Specific Are the Tests?

Limited data are available. MAb T14 has demonstrated no cross-reactivity with *Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*, *V. cholera*, *E. coli, S. typhimurium, Fr. novicida, Br. melitensis, Br. abortus, Br. suis, Br. ovis,* or *Br. neotomae*. MAb FB11 has demonstrated no cross-reactivity with *Fr. novicida, Br. abortus, Br. suis, Br. melitensis, Br. ovis, Y. pestis, Y. enterocolitica, Y. pseudotuberculosis, E. coli,* or *V. cholerae*. These antibodies are largely used for Enzyme Immunoassay (EIA), immunofluorescence.

Helicobacter pylori

32.15 How Sensitive Are the Tests?

IHC has proved superior to routine histochemistry, but results have varied. In a study of 48 cases, *H.pylori* was demonstrated by both techniques in 27. In 2 cases, the immunostain could not demonstrate the bacteria but they were identified with a modified Giemsa stain. In 5 cases, the bacteria were identified by the immunostain but not with the modified Giemsa stain [15]. In another study, bacteria were detected in 66 % of tissue sections stained with the antibody. This compared favorably to silver stains and PCR [16]. In other studies, PCR and ISH have proved superior [17].

32.16 How Specific Are the Tests?

Using culture as the gold standard, specificity was 90 % and sensitivity was 83.8, compared with 53.8 and 90 %, respectively for modified Geimsa and 82.5 and 70 %, respectively for a Warthin-Starry stain [18].

32.17 Does Fixation Affect the Test?

Depending upon the fixation method and the staining system employed, optimal incubation conditions may vary. Formalin-fixed paraffin embedded tissue sections require high temperature antigen unmasking in 10 mM citrate buffer, at pH 6.0, although this may vary by antibody system.

Leptospirosis

32.18 How Sensitive Are the Tests?

Some data suggest that immunohistochemistry does not enhance sensitivity compared to silver staining, it improved the ease of diagnosis [19]. One studies demonstrated 78 % sensitivity [20].

32.19 How Specific Are the Tests?

Specificity appears as high as 100 %.

32.20 Does Fixation Affect the Test?

Some antibody systems require sections to be treated with trypsin. Antigen retrieval may be performed on slides preheated to 37 C by microwaving for 1.5 min at 630 W followed by 5 min at 180 W in Tris (pH 10) buffer, although the product insert should be consulted for the antibody system used.

Lyme Disease

32.21 How Sensitive and Specific Are the Tests?

IHC has demonstrated sensitivity as high as 96 % with specificity of 99.4 %, compared to 45.5 % sensitivity and 100%specificity for PCR run on the same tissue. Other authors have found a sensitivity of only 39 % and suggested that because the density of *B. burgdorferi* in human tissue is very low, the method is not useful in a clinical setting [21]. The technique pioneered by Bernhard Zelger of Innsbruck/Austria requires 100× oil immersion in conjunction with "native" (i.e. no blue counterstain) immunostained sections—and lots of time to look for organisms.

32.22 Does Fixation Affect the Test?

Some antibody systems require trypsin.

Pseudomonas aeruginosa

Culture remains the gold standard. Identification of the bacilli in formalin-fixed tissues can be performed using IHC and PCR assays [22]. In addition to specific monoclonal antibody staining, the anti-BCG stain has been used.

32.23 How Sensitive and Specific Are the Tests?

Limited data are available.

32.24 Does Fixation Affect the Test?

Choice of fixation has not shown a significant effect on results to date.

Rickettsia rickettsii/Rocky Mountain spotted fever (RMSF)

32.25 How Sensitive Are the Tests?

In one study, both immunoperoxidase staining and immunofluorescence detected the organism in 9 of 10 specimens [23]. Antibodies are available to detect spotted fever group and typhus group organisms.

32.26 How Specific Are the Tests?

It may not be possible to distinguish between spotted fever group organisms.

32.27 Does Fixation Affect the Test?

Antigen retrieval varies by antibody system.

Treponema pallidum

32.28 How Sensitive Are the Tests?

In one study, immunohistochemistry testing was positive in 17/35cases, compared with 9/35 for Dieterle staining and 14/36 for PCR [24]. Other studies have shown from 71 to 91 % sensitivity in patients with secondary compared with 41 % using a silver stain [25, 26]. When the index of suspicion is high and staining is negative, the immunostain should be repeated or a silver stain performed [27].

32.29 How Specific Are the Tests?

Specificity is higher than with silver staining, but more data are needed regarding cross reaction with other spirochetes. Cross reactivity with *M. leprae* has been reported [28].

32.30 Does Fixation Affect the Test?

The effect of fixation on IHC may be pH sensitive. Both immunohistochemisty and PCR require neutrally buffered formalin. Acid destroys DNA and epitopes.

Yersinia pestis

Culture remains the gold standard. Identification of the bacilli in formalin-fixed tissues can be performed using IHC, DFA, and PCR assays [29].

32.31 How Sensitive and Specific Are the Tests?

Limited data are available.

32.32 Does Fixation Affect the Test?

Choice of fixation has not shown a significant effect on results to date.

32.33 How Should I Incorporate These Tests into My Practice?

IHC has become standard for the detection of *Treponema pallidum* (*T. pallidum*). It still suffers from limited sensitivity, and silver stains should be performed if there is a high degree of suspicion and the immunostain is negative. IHC is being used in some laboratories for the detection of *Rickettsia* and *Chlamydia*. Testing for anthrax and other bioterrorism agents is likely to be performed by the CDC. Silver staining remains the most sensitive test for *B. henselae,* but is the least specific. IHC staining suffers from low sensitivity, but is useful for confirmation of the diagnosis because of its high specificity. PCR remains helpful as a second-line test for IHC negative cases. Although culture remains the gold standard for the detection of *B. melitensis*, IHC can be used to demonstrate the presence of *B. melitensis* antigens in tissue sections.

Figures 32.1, 32.2, 32.3, and 32.4.

Table 32.1.

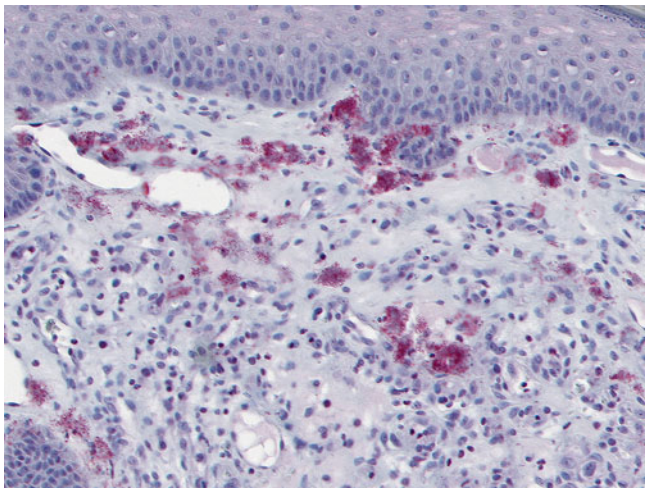

Fig. 32.1 Bacillary angiomatosis (*Bartonella henselae)* IHC ×200

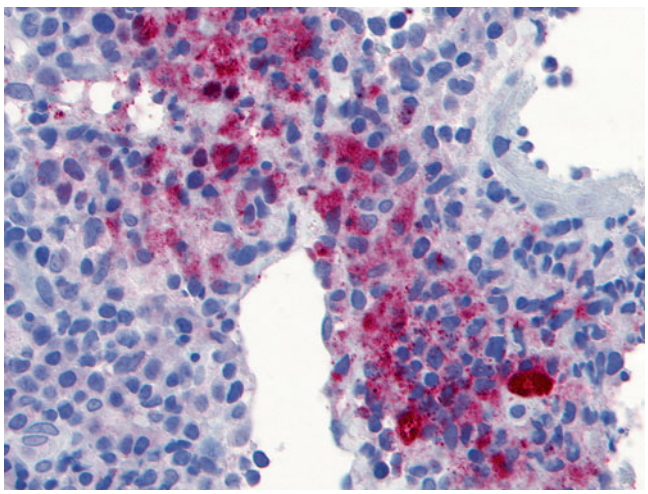

Fig. 32.2 Lymphogranuloma venereum (*Chlamydia trachomatis*) ×400

32.34 What Tests Are Available to Detect Mycobacteria?

Culture, cutaneous tuberculin testing and interferon gamma release assays remain the gold standards in the diagnosis of tuberculosis. Auramine-Rhodamine staining is extremely sensitive, but requires a fluorescent microscope. For this reason,

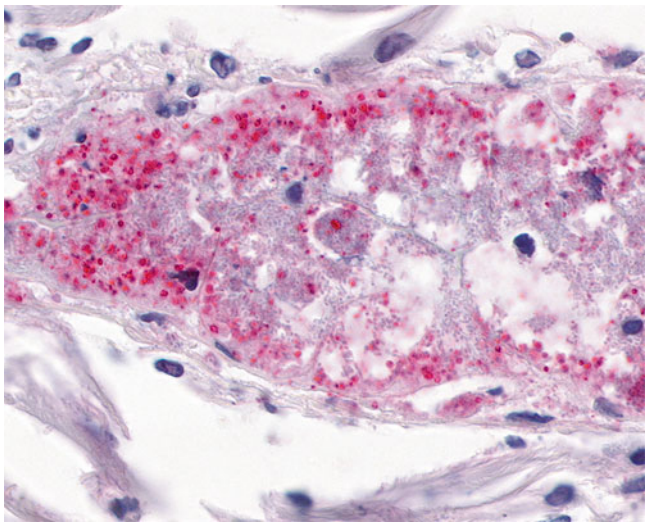

Fig. 32.3 Ecthyma gangrenosum (*Pseudomonas* sepsis) anti-BCG stain IHC ×400

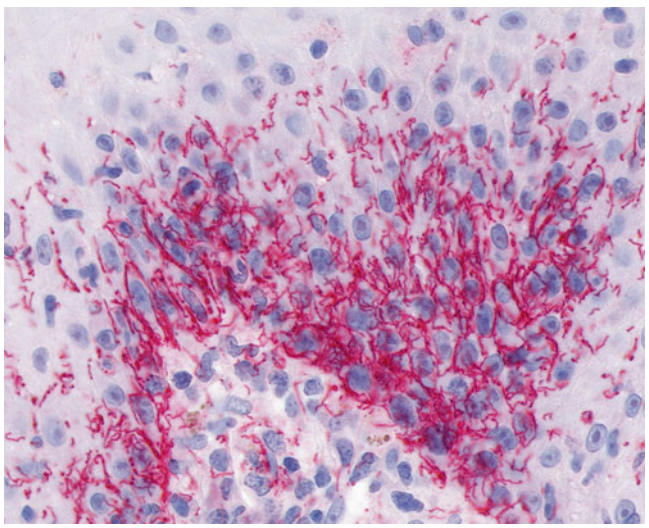

Fig. 32.4 Syphilis (*Treponema pallidum)* ×400

Table 32.1 Commonly used immunohistochemical stains for the detection of bacteria

Stain	Comment
B. henselae	Perform silver stain if negative and clinical suspicion high
Rickettsia	Immunostains for spotted fever and typhus groups
Treponema pallidum	Perform silver stain if negative and clinical suspicion high

routine histochemical staining is still commonly performed and IHC has a place in the diagnostic armamentarium.

Anti- bacille Calmette-Guerin (BCG) Antibody Immunostain for Acid Fast Bacteria and Fungi

32.35 How Sensitive Are the Tests?

Strong or moderate positive reactions are almost always observed for fungi. A wide variety of bacterial and protozoan species are positive. In one study, 4 protozoan and 12 bacterial species, including *Leptospira* and *Mycoplasma* were negative. Overall, IHC showed similar sensitivity to bacteriological culture and was more sensitive than routine histochemisty [30]. It has been used to detect atypical mycobacteria to include *M. abscessus* [31]. In the setting of leprosy, it has proved more sensitive than routine histochemical staining.

32.36 How Specific Are the Tests?

In the setting of early leprosy, false-positive staining was noted in 16 % of patients [32]. IHC testing for BCG is quite nonspecific in regard to the identity of the organism, as the antibody reacts with many bacteria, fungi and protozoa in formalin-fixed paraffin-embedded tissue samples.

32.37 Does Fixation Affect the Test?

Heating of the sections in a microwave oven is generally the most effective method.

Mycobacterium avium subsp. Paratuberculosis

32.38 How Sensitive Are the Tests?

Sensitivity of some antibodies may be as low as 5 % compared to culture, while others may achieve 93 % sensitivity [33, 34].

32.39 How Specific Are the Tests?

Limited data are available.

32.40 Does Fixation Affect the Test?

Limited data are available.

Mycobacterium Leprae

In addition to the BCG stain described above, immunolabeling of lipoarabinomanan (LAM) and/or phenolic glycolipid 1 (PGL-1) has been used to detect *M. leprae.* PCR assays are also available [35].

32.41 How Sensitive Are the Tests?

In the setting of pure neural leprosy, the stain performed slightly better than the existing PCR assay, staining 8 of 17 specimens [36].

32.42 How Specific Are the Tests?

Limited data are available.

32.43 Does Fixation Affect the Test?

Limited data are available.

Mycobacterium tuberculosis and M. bovis

IHC has been used to detect tuberculosis organisms in tissue. One antibody targets the secreted mycobacterial antigen MPT64, and has been used in formalin-fixed tissue biopsies [37].

32.44 How Sensitive and Specific Are the Tests?

With IHC 64 % (35/55) and with PCR, 60 % (33/55) of cases granulomatous lymphadenitis were positive in one study. Compared to PCR, immunohistochemistry demonstrated sensitivity, specificity, positive and negative predictive values of 90, 83, 86, and 88 %, respectively [38]. In another study, acid fast bacilli were observed in only 36.1 % of tuberculous granulomas with routine histochemical staining while immuno-histochemical staining was positive in 100 % with no false positives [39].

32.45 Does Fixation Affect the Test?

Limited data are available.

32.46 How Should I Incorporate These Tests into My Practice?

The BCG antibody is very useful as a screening method to detect a wide variety of pathogens, and is especially useful when pathological features suggest an infection, but no microorganism can be cultured or only formalin-fixed tissue samples are available. Specific IHC testing for mycobacteria is not yet in widespread clinical use. A cocktail of mycobacterial and cross-reacting antibodies developed by Cristina Riera from Barcelona includes anti-mycobacterium bovis BCG, anti-mycobacterium tuberculosis, and anti-leishmaniasis antibodies. It is currently receiving good reviews from some labs.

Figures 32.5, 32.6, and 32.7
Table 32.2.

32.47 What Tests Are Available to Detect Viruses?

Serologic studies, in situ hybridization (ISH), polymerase chain reaction (PCR) and direct fluorescent antibody assay (DFA) are widely used for the diagnosis of viral diseases. Immunohistochemistry (IHC) is used to detect a number of viruses in veterinary medicine, but it has become standard only for a few in humans.

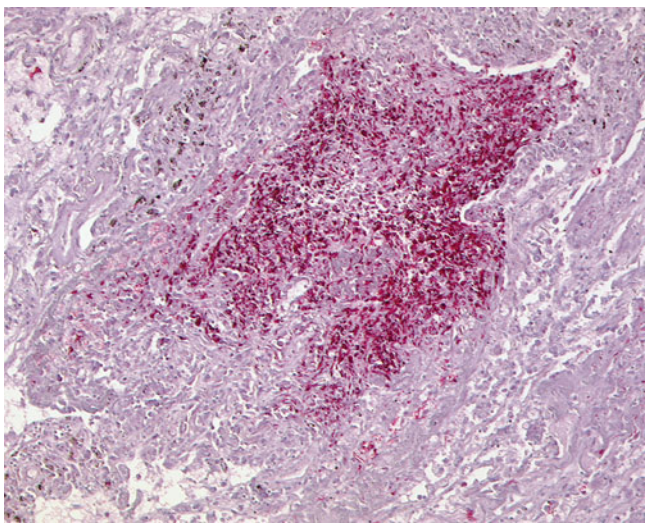

Fig. 32.5 Tuberculosis, lung IHC ×100

Cytomegalovirus (CMV)

32.48 How Sensitive and Specific Are the Tests?

In one study, IHC detected the virus in only five of nine patients [40]. Another study showed detection of virus in 23 of 36 tissue samples, number comparable to that seen with ISH. PCR is superior to both methods if fresh tissue is available, but each method may detect some infections the others fail to detect [41–43]. With newer methods, IHC achieves sensitivities of 75.7 % with a specificity of 100 % [44].

32.49 Does Fixation Affect the Test?

Formalin fixation decreases the sensitivity of IHC, but may have an even greater influence on PCR results. In general, we have had very positive experience with the antibody regardless of fixation.

Enterovirus Infection

IHC has been used to detect viral particles in the setting of hand foot and mouth disease.

32.50 How Sensitive Are the Tests?

Data are limited.

32.51 How Specific Are the Tests?

Data are limited.

32.52 Does Fixation Affect the Test?

Data are limited.

Epstein-Barr virus (EBV)

In situ hybridization remains the gold standard for EBV detection in tissue. While IHC has also been used, it is a poor second choice [45].

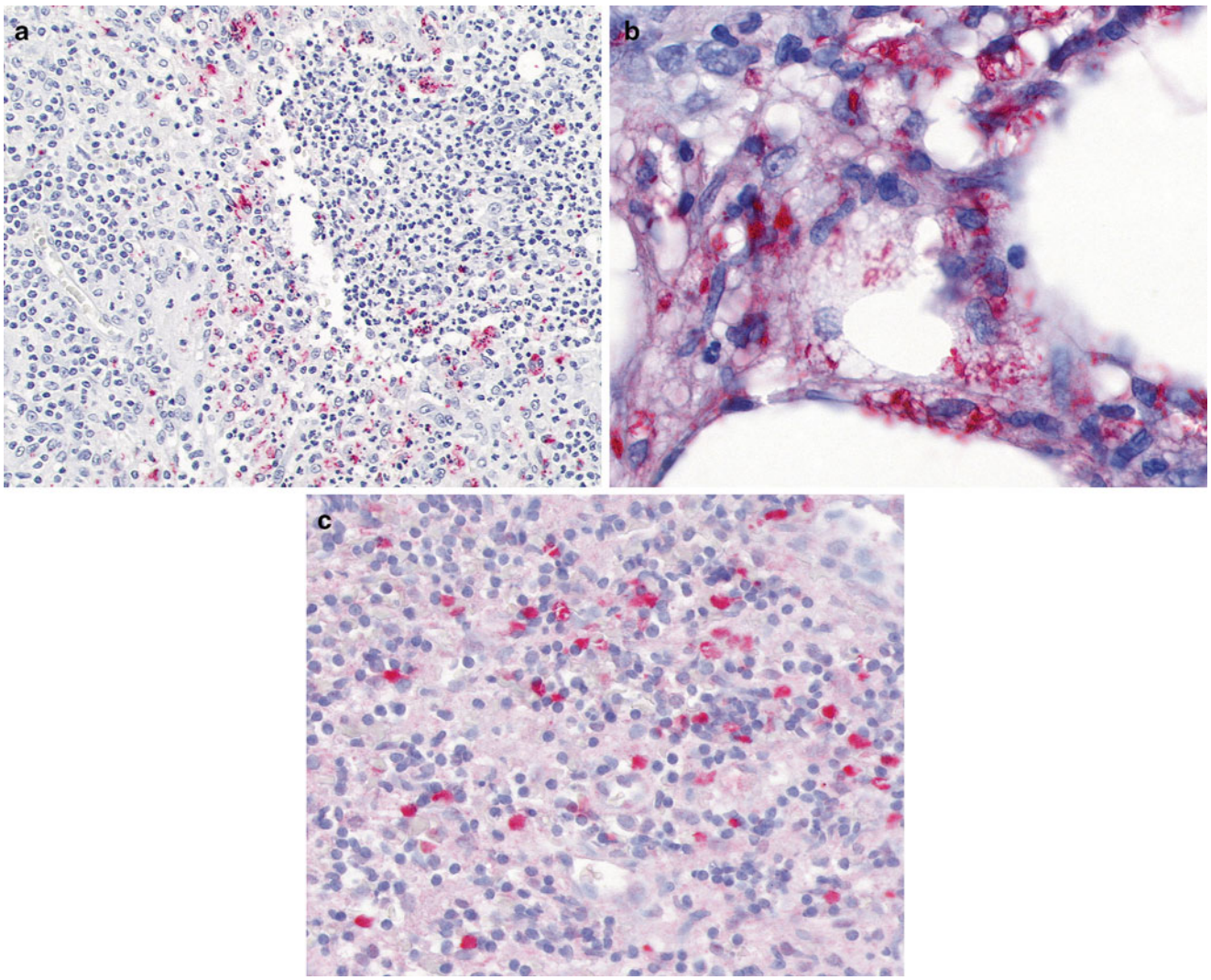

Fig. 32.6 (**a**) Atypical mycobacterial panniculitis IHC ×100, (**b**) Atypical mycobacterial panniculitis IHC ×400, (**c**) *Mycobacterium avium silvaticum* IHC ×100

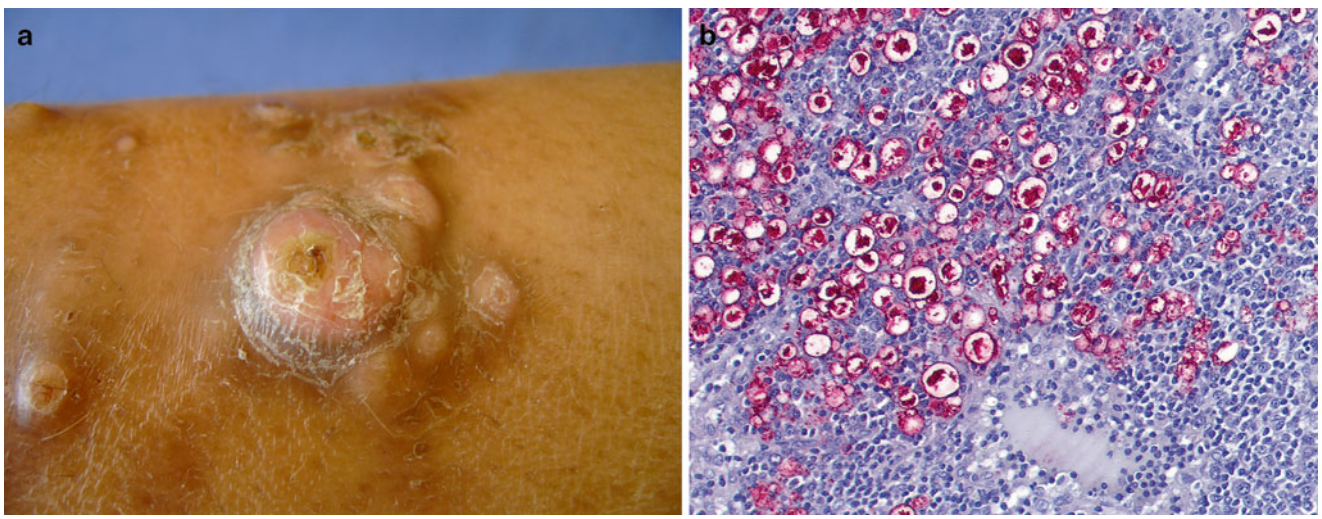

Fig. 32.7 (**a**) Lepromatous leprosy, (**b**) Lepromatous leprosy IHC ×100

Table 32.2 Commonly used immunohistochemical stains for the detection of mycobacteria

Stain	Comment
Anti-*BCG*	Broad screen for many mycobacteria, bacteria, and fungi
Mycobacterium avium subsp. paratuberculosis	Culture remains the gold standard
Mycobacterium tuberculosis and *M. bovis*	Culture, tuberculin testing and interferon gamma release assays remain the gold standard

32.53 How Sensitive and Specific Are the Tests?

In one study, automated IHC had a sensitivity of 44 % and specificity of 93 %. In comparison, ISH achieved a sensitivity and specificity of 94 % and 69 %, respectively [46]. In some settings, PCR testing can produce results similar to ISH [47], but PCR detection is problematic as much of the population has been infected and a positive result may not correspond to causation of the lesion being studied.

32.54 Does Fixation Affect the Test?

Comparable staining has been noted with formalin and methanol fixation [48].

Hepatitis C

32.55 How Sensitive and Specific Are the Tests?

Compared to the serology, 83 % and 67 % of the cases were positive with immunohistochemistry and in situ RT-PCR respectively [49]. We have found many false negatives with the technique. In another study, 16 of 20 serum antibody-positive cases were detected with IHC, compared with 18 with RT-PCR and 19 with ISH [50]. Using a five-step peroxidase-antiperoxidase method, hepatitis C virus core and non-structural four antigens were detected in 71 % and 57 % of patients, respectively [51]. Although IHC testing lacks sensitivity compared to other methods, it has the advantage of localizing the virus in tissue.

32.56 Does Fixation Affect the Test?

Data are limited.

Human Herpes Virus Type 6 (HHV-6)

32.57 How Sensitive Are the Tests?

With a modified avidin-biotin complex (ABC) method, staining for HHV-6 was noted in six of eight patients [52].

32.58 How Specific Are the Tests?

Data are limited. In our experience, currently available HHV-6 and HHV-7 antibodies are of limited usefulness.

32.59 Does Fixation Affect the Test?

Data are limited.

Human Herpes Virus Type 8 (HHV-8)

32.60 How Sensitive and Specific Are the Tests?

IHC using a monoclonal antibody to human herpes virus 8 latent nuclear antigen-1, achieves sensitivity and specificity as high as 100 % in paraffin-embedded tissue sections of Kaposi sarcoma [53].

32.61 Does Fixation Affect the Test?

The test works reliably in formalin-fixed tissue.

Human Papillomavirus (HPV)

ISH is used more commonly in this setting and PCR with sequencing is also used.

32.62 How Sensitive Are the Tests?

HPV-L1 capsid antibody can be helpful but does not detect nuclear HPV DNA. Combined with p16 staining, it can increase the sensitivity of detection of HPV infection in gynecological specimens [54]. There are anti-HPV16 antibodies available for IHC.

32.63 How Specific and Sensitive Are the Tests?

Monoclonal antibodies to specific HPV types are available, and some have shown excellent sensitivity and specificity [55]. HPV 6 and 11 are the predominant viruses associated with condyloma and tend to remain benign. Other HPV types (e.g. 16/18/31/33/35/39/45/51/52/56/58/66) are more closely associated with cervical cancer. ISH may be of help if morphologic changes of viral infection are present or suspected and positive identification is clinically relevant for risk management or priority of treatment. A negative test does not rule out presence of HPV as many HPV types are not detected by these tests to date. IHC for p16(INK4a), a marker of cell cycle dysregulation, is used as a surrogate marker for HPV in cervical dysplasias and carcinomas associated with high risk (HR-HPV) infections. As a surrogate marker, it shows greater specificity than sensitivity and is best used as a screening tool [56]. While p16 expression is commonly used in cervix, it plays little role in the evaluation of skin specimens.

32.64 Does Fixation Affect the Test?

The tests work well in formalin fixed tissue.

HSV

DFA, culture and serologic assays are used much more commonly than IHC. In situ hybridization tests have also been developed for examination of formalin-fixed, paraffin embedded tissue.

32.65 How Sensitive Are the Tests?

DFA and ISH have high sensitivity and specificity. PCR is more sensitive than IHC for detection of herpes simplex virus. In one study, the former was positive in approximately 90 % of patients and the latter in approximately 50 % [57].

32.66 How Specific Are the Tests?

IHC studies are used less often, but some antibodies produce very reproducible results in the lab. It should be noted that types 1 and 2 cross react. ISH is highly specific if viral genome is present.

32.67 Does Fixation Affect the Test?

Data are limited. ISH likely performs better in tissue fixed 24 h or less in formalin.

Influenza A virus

Worldwide outbreaks of H1N1 swine influenza and H5N1avian influenza have highlighted the need to develop better tests for detection of influenza A virus in tissue. The H5-specific monoclonal antibody 7H10 has been used for immunohistochemical staining in formalin-fixed tissue. DFA and ELISA assays are also used.

32.68 How Sensitive Are the Tests?

IHC using 7H10 detected 28 of the 29 H5 virus strains tested, and, the eight-residue-long linear epitope, FFWTILKP, allowed 7H10 to detect >98.3 % of H5 subtypes reported before 2007 [58].

32.69 How Specific Are the Tests?

None of non-H5 strains were detected by 7H10.

32.70 Does Fixation Affect the Test?

Data are limited.

Ljungan Virus

Ljungan virus (LV), a viral pathogen implicated in fetal death, can be detected by IHC and PCR.

32.71 How Sensitive Are the Tests?

LV was demonstrated in 5 of 5 cases by IHC and confirmed 3 of 5 by real time RT-PCR [59].

32.72 How Specific Are the Tests?

Only one of 18 control specimens was positive by IHC.

32.73 Does Fixation Affect the Test?

Can be performed on formalin fixed tissue, but optimal retrieval methods remain to be determined.

Parvovirus B19

Parvovirus B19 infection is implicated in fifth disease, purpuric gloves and socks syndrome, adult arthritis syndrome, aplastic crisis, dilated cardiomyopathy, and fetal death from hydrops fetalis. Serologic assays, IHC, PCR and ISH are often used to confirm the presence of the virus [60, 61].

32.74 How Sensitive and Specific Are the Tests?

Compared to PCR results, the sensitivity of anti-B19V IHC in the setting of dilated cardiomyopathy was 80.0 %, and the specificity was 86.0 % [62].

32.75 Does Fixation Affect the Test?

Data are limited.

Rabies

Fluorescent and PCR assays are used more commonly than IHC.

32.76 How Sensitive Are the Tests?

An indirect immunoperoxidase technique (VNT-IIP) showed high sensitivity (92.8 %) and specificity (87.0 %) when compared with the fluorescent antibody virus neutralization test [63]. A direct rapid immunohistochemical test (dRIT) demonstrated 100 % sensitivity and specificity compared to the direct fluorescent antibody test in field testing [64].

32.77 How Specific Are the Tests?

Antibodies against rabies may cross react with Duvenhage virus, Mokola virus and European bat lyssavirus-1 [65].

32.78 Does Fixation Affect the Test?

For analysis, a piece of fresh brain tissue should preferably be stored no longer than 24 h in formalin before embedding [66].

Small Pox (Variola)

Although both IHC and DFA have demonstrated the virus in a variety of tissues, including skin, liver and fibroconnective tissue, fluorescent assays are used almost exclusively.

32.79 How Sensitive Are the Tests?

Fluorescent assays have shown excellent sensitivity and specificity. A rapid, sensitive real-time assay to detect *Variola* was developed using the *Vaccinia* virus as a target [67].

32.80 Does Fixation Affect the Test?

Data are limited.

Varicella-Zoster Virus (VZV)

As with HSV, PCR and DFA are used much more commonly than IHC [68]. ISH is also available for VZV.

32.81 How Sensitive and Specific Are the Tests?

In one study, immunohistochemistry achieved a type-specific virus diagnostic accuracy of between 86.7 % and 100 % on smears, and 92.3 % in skin sections [69]. Shell vial immunoperoxidase has demonstrated 87.6 % sensitivity and 100 % specificity when compared with cell culture [70]. ISH specificity is 100 % if viral genome is present in the tissue.

32.82 Does Fixation Affect the Test?

Limited data are available but ISH performs better in tissue fixed in formalin for 24 h or less.

Viral Hemorrhagic Fevers

Hemorrhagic fever virsuses include the *Filoviridae* (Ebola and Marburg viruses) and the *Arenaviridae* (Junin, Machupo, Guanarito, and Lassa viruses). These can be detected using PCR, IHC, or electron microscopy. During outbreaks of Ebola hemorrhagic fever in Africa, IHC was used successfully on skin punch biopsy samples in large numbers of fatal cases [71]. These tests are not generally available.

West Nile virus (WNV)

The diagnosis is usually made via serologic studies. A variety of antibodies are available including a rabbit-polyclonal anti-WNV antibody and a monoclonal antibody directed against an epitope on Domain III of the E protein of WNV.

32.83 How Sensitive Are the Tests?

In studies on the kidney, liver, lung, spleen, and small intestine of infected crows, the sensitivity of the monoclonal antibody-based IHC staining was only 72 %, compared to 100 % with the polyclonal antibody [72]. In human tissue, the concordance between IHC and serology was 41 %, while the concordance between RT-PCR and serology was 63 % [73].

32.84 How Specific Are the Tests?

Data are limited.

32.85 Does Fixation Affect the Test?

Data are limited.

32.86 How Should I Incorporate These Tests into My Practice?

IHC is commonly used for the detection of HHV-8, and is also used for CMV. Other techniques, such as DFA are widely used for other herpes viruses. ISH studies are commonly performed for HPV. Detection of HSV or VZV vial changes can most often be done morphologically on routinely stained sections. However, in cases with atypical presentations or where specific rapid differentiation is required for therapeutic purposes, ISH may play a role due to its specificity. Specialized laboratories, such as those at the CDC will perform a wider range of testing, including IHC for exotic viruses and those likely to be used as biological weapons.

Figures 32.8, 32.9, 32.10, 32.11, 32.12, 32.13, 32.14, and 32.15.
Table 32.3.

32.87 What Tests Are Available to Detect Fungal and Protozoan Pathogens?

Anti-Bacille Calmette-Guerin (BCG) Antibody Immunostain

Specific IHC tests are being developed, but the anti-bacille Calmette-Guerin (BCG) antibody immunostain has been the most common stain in use. DAKO recently modified

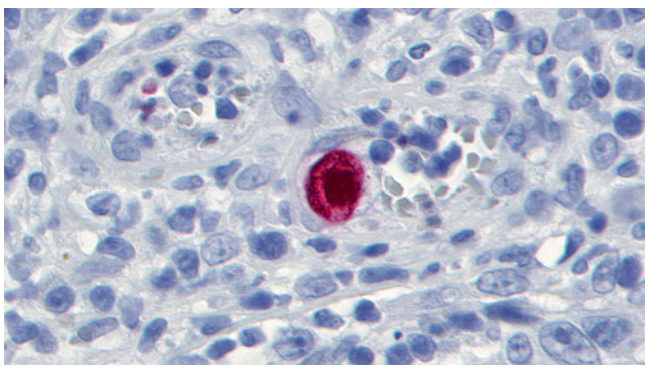

Fig. 32.8 Cytomegalovirus (CMV) ×600

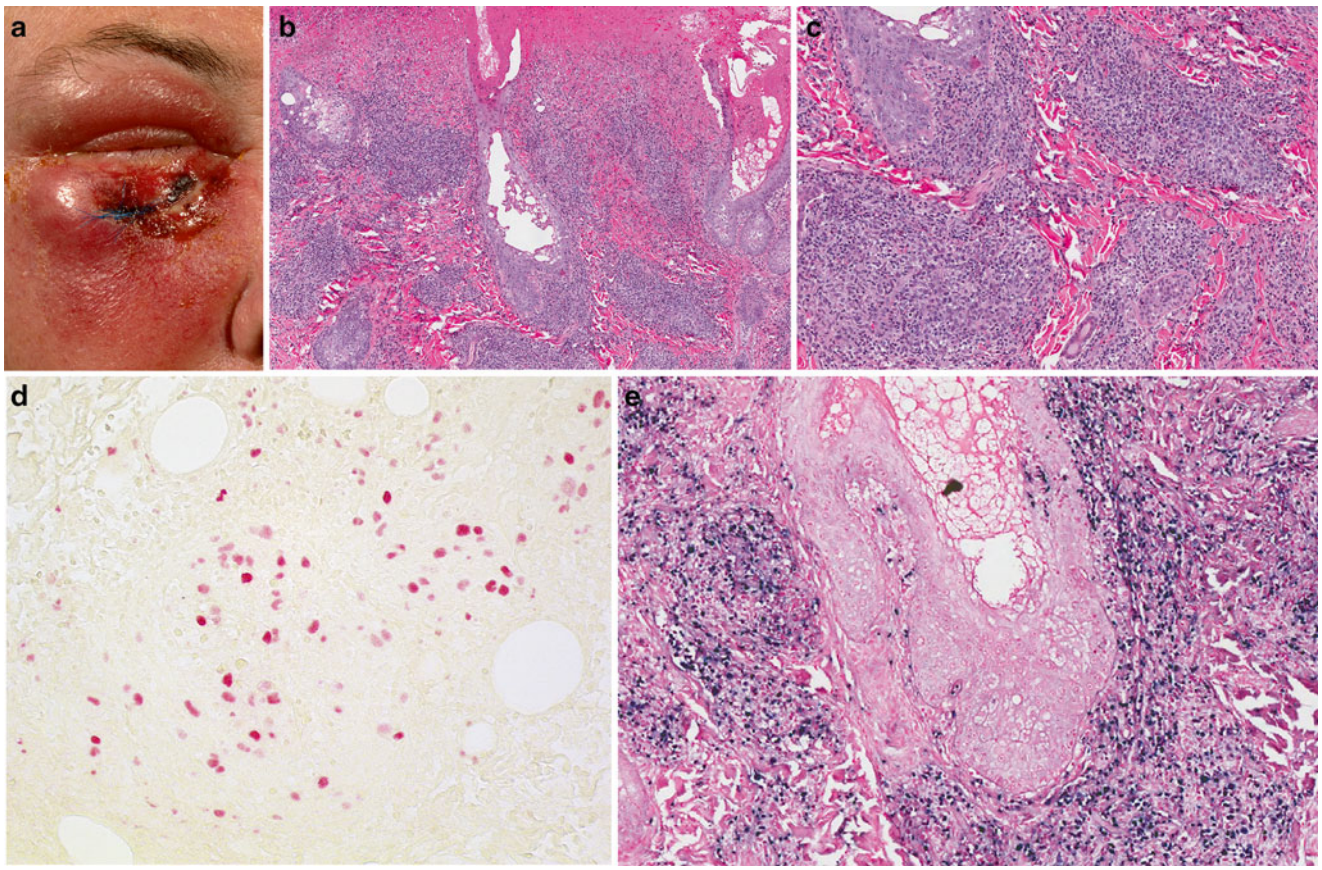

Fig. 32.9 (**a**) Epstein-Barr virus (EBV), (**b**) Epstein-Barr virus (EBV) H&E ×40, (**c**) Epstein-Barr virus (EBV) H&E ×80, (**d**) Epstein-Barr virus (EBV) ISH ×40, (**e**) Epstein-Barr virus (EBV) ISH ×100

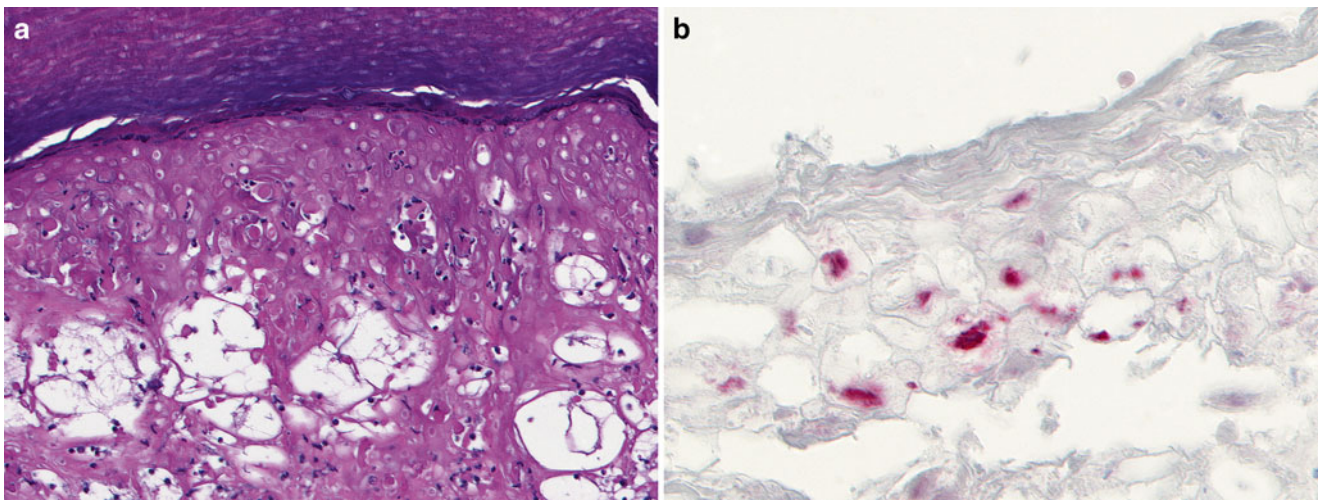

Fig. 32.10 (**a**) Hand foot and mouth syndrome H&E ×200, (**b**) Hand foot and mouth syndrome IHC ×400

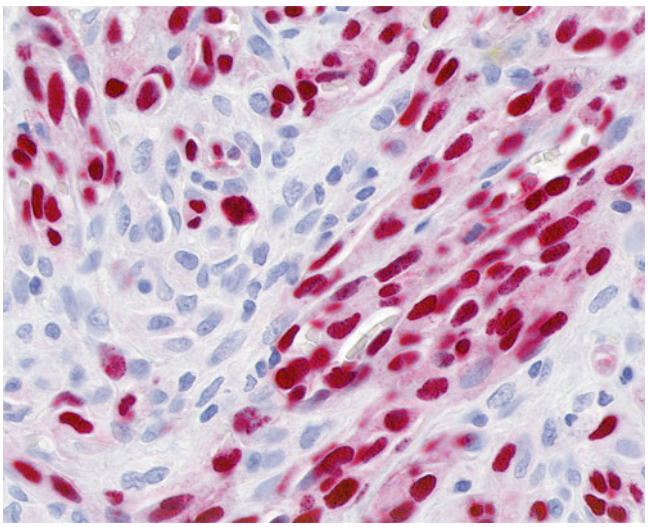

Fig. 32.11 Human herpes virus 8 (HHV8) IHC ×400

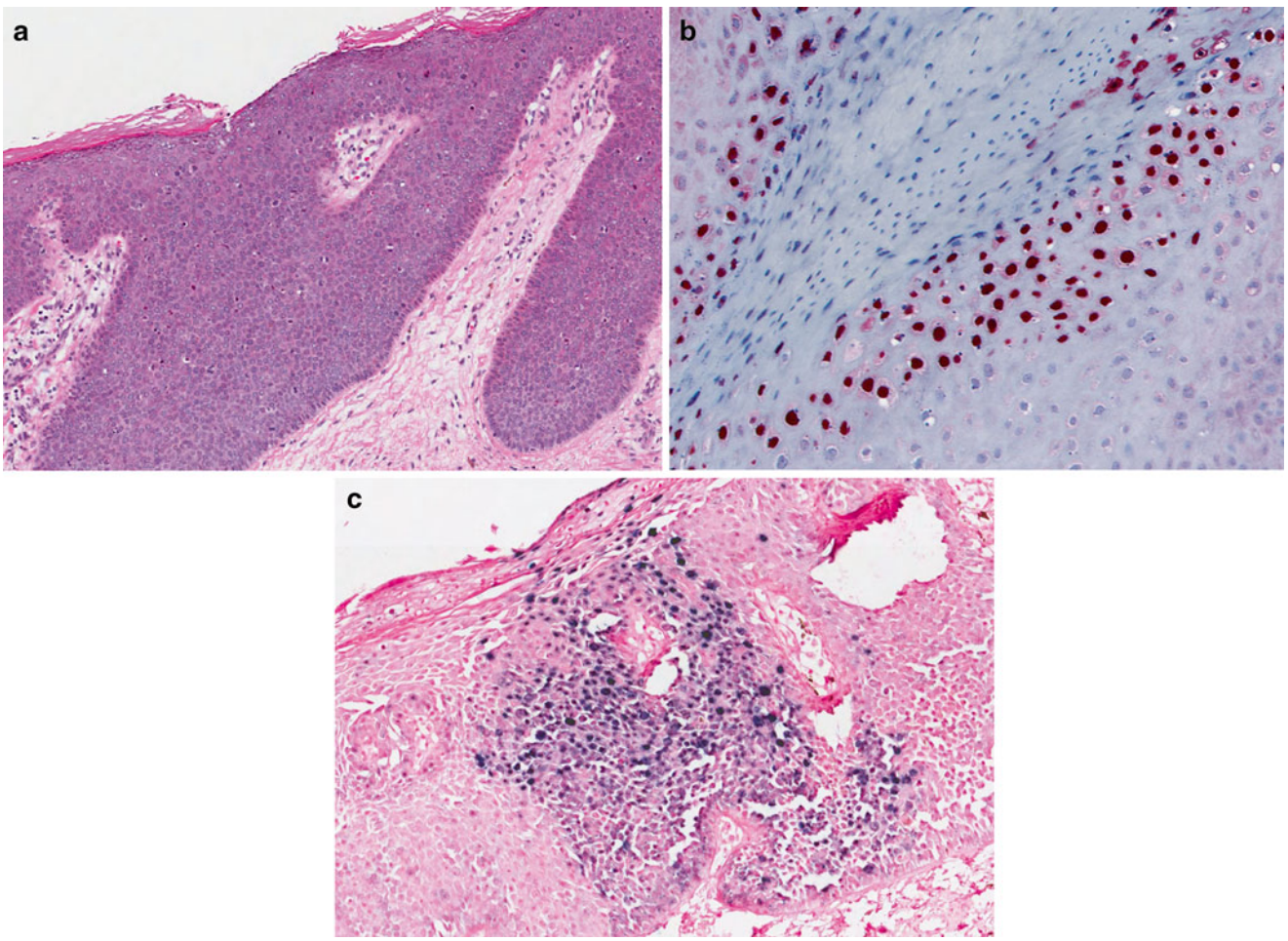

Fig. 32.12 (**a**) Human papilloma virus (HPV) H&E ×80, (**b**) Human papilloma virus (HPV) IHC ×200, (**c**) Human papilloma virus (HPV) ISH ×100

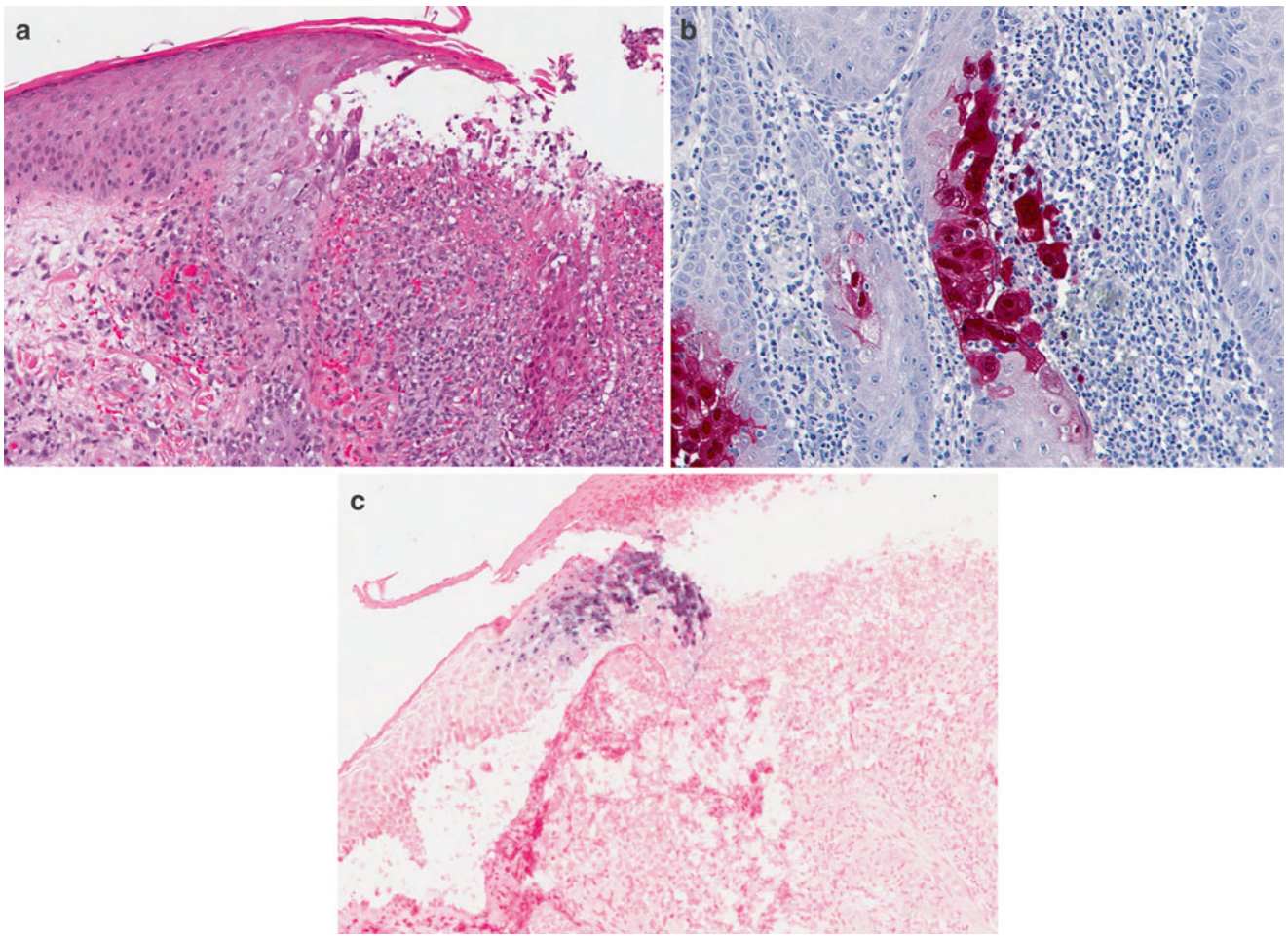

Fig. 32.13 (**a**) Herpes simplex virus (HSV) H&E ×100, (**b**) Herpes simplex virus (HSV) IHC ×200, (**c**) Herpes simplex virus (HSV) ISH ×80

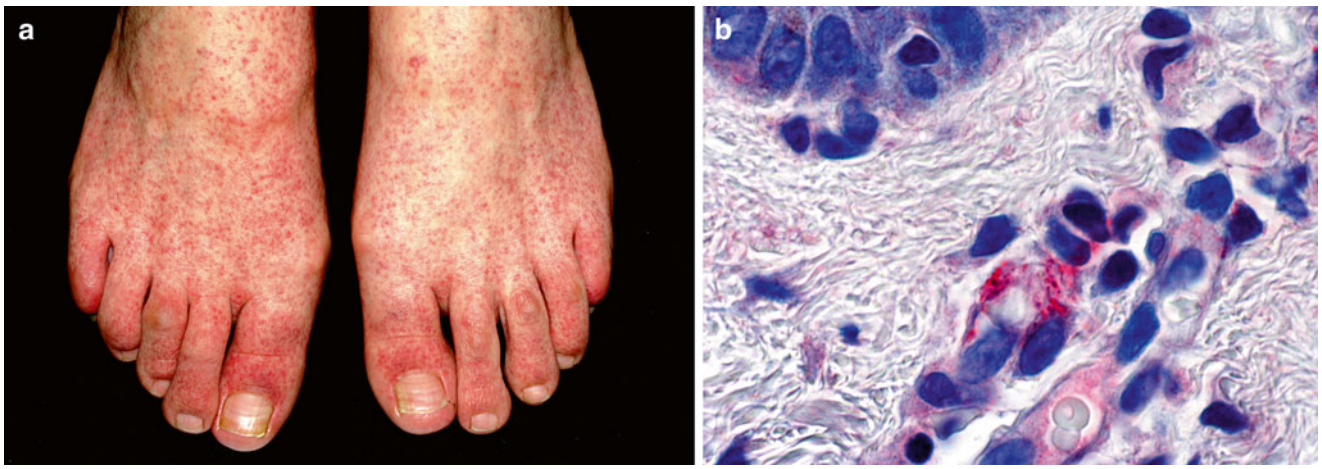

Fig. 32.14 (**a**) Parvovirus B19, purpuric gloves and socks syndrome, (**b**) Parvovirus B19 IHC ×600

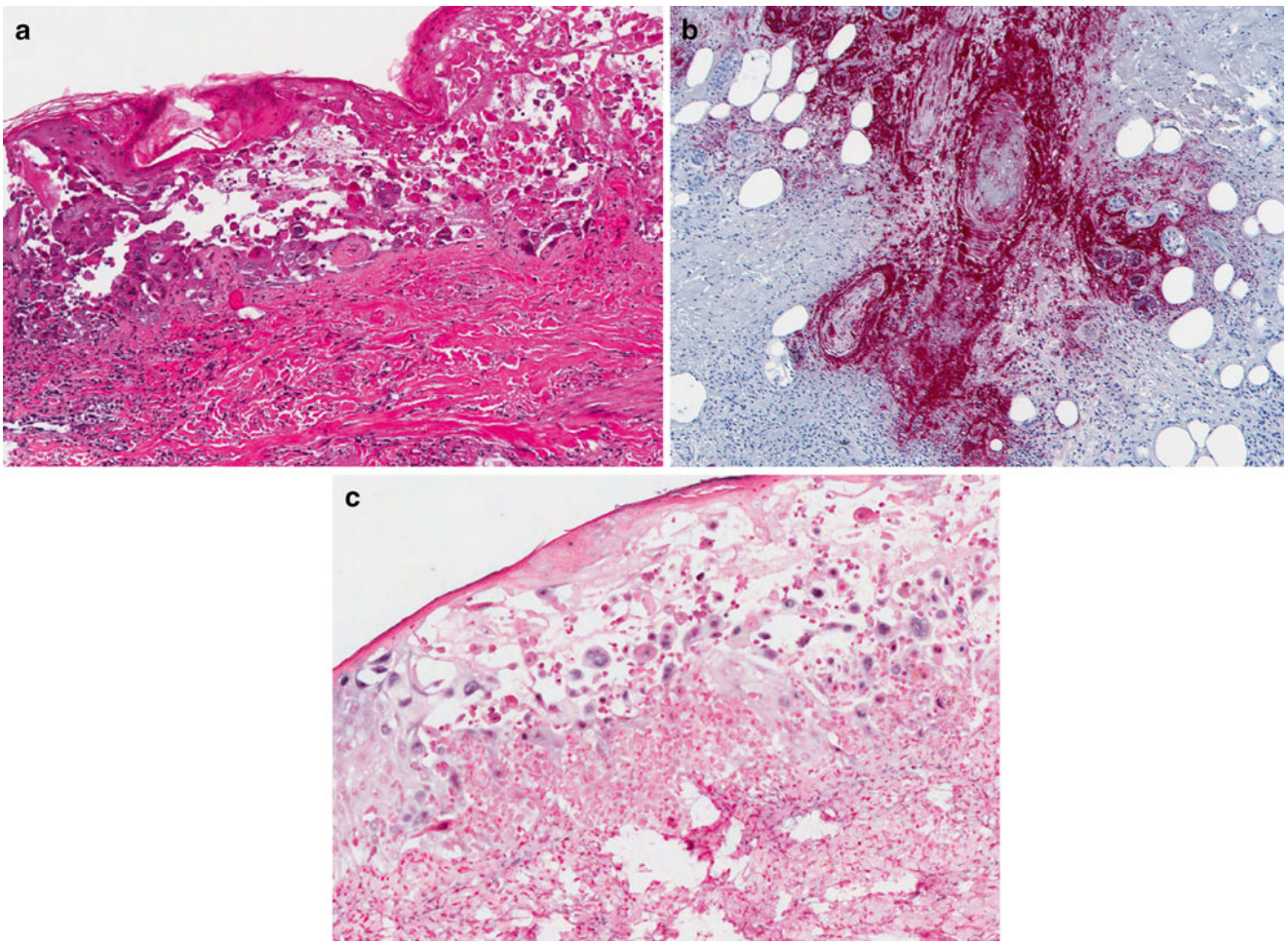

Fig. 32.15 (**a**) Varicella zoster virus (VZV) H&E ×100, (**b**) Varicella zoster virus (VZV) IHC ×200, (**c**) Varicella zoster virus (VZV) ISH ×100

Table 32.3 Commonly used immunohistochemical stains for the detection of viruses

Stain	Comment
CMV	PCR is superior to IHC and ISH if fresh tissue is available
HHV-8	High sensitivity and specificity

their antibody, and it has lost reactivity with fungal species. PCR and ISH assays are also commonly used and isolation by culture remains the gold standard.

32.88 How Sensitive Are the Tests?

Results vary by the organism. PCR is more sensitive than IHC using a BCG immunostain for the detection of *Histoplasma capsulatum*. In one study, the 50 % quantile to achieve a positive result for each study was determined to be 3 colony-forming units per milligram for PCR, 11 for Grocott stain, 27 for a fluorochrome stain, 190 for immunostaining, and 533 for H&E [74].

32.89 How Specific Are the Tests?

IHC testing for BCG is quite nonspecific in regard to the identity of the organism, as the antibody reacts with many bacteria, fungi and protozoa in formalin-fixed paraffin-embedded tissue samples.

32.90 Does Fixation Affect the Test?

The test works reliably in formalin-fixed tissue.

Aspergillus Species

The monoclonal antibody EB-A1 has been used to detect *Aspergillus species* in formalin fixed,paraffin wax embedded tissue.

32.91 How Sensitive Are the Tests?

IHC staining was positive in 89 % of cases, including one culture negative case with histological evidence of infection [75]. Polyclonal and monoclonal *Aspergillus* antibodies have shown sensitivities and specificities of 100 % and 29 %, and 43 % and 14 %, respectively. Cross reaction with zygomycetes was noted [76].

32.92 How Specific Are the Tests?

Cross-reactivity was observed with *Pseudallescheria boydii,* but not with *Candida* species, *Apophysomyces elegans*, *Rhizopus oryzae*, or *Histoplasma capsulatum.* Polyclonal antibodies have shown a high degree of cross reactivity with other fungi [77].

32.93 Does Fixation Affect the Test?

Limited data are available.

Blastomyces dermatitidis, Coccidioides immitis, Cryptococcus neoformans and Histoplasma capsulatum

Identification of these yeast-like organisms is of importance particularly in the transplant and immunocompromised patient populations. Most often these organisms can be identified in tissue sections utilizing silver stains (e.g. GMS) or the PAS stain. However in cases where rapid identification of specific organism is required or in cases where the possibility of more than one infection exists, there is a role for in situ hybridization (ISH) diagnosis. Specific probes designed to detect ribosomal RNA sequences to several fungal organisms including all of the above have been developed.

32.94 How Sensitive Are the Tests?

Sensitivity for detection of yeast or hyphal forms is slightly less than traditional silver or PAS stains (83 % vs 95 %).

32.95 How Specific Are the Tests?

Specificity is very high if organisms are present in tissue (100 %) vs 96 to 100 % utilizing traditional silver stains or PAS [78, 79].

32.96 Does Fixation Affect the Test?

Formalin-fixed, paraffin embedded tissue may be used. It is recommended, however, that the tissue not be fixed in formalin greater than 24 h before processing and embedding.

Pneumocystis carinii

A monoclonal antibody has been developed that recognizes *Pneumocystis carinii* in tissue, bronchoalveolar lavage fluid, and sputum. The antibody has been adapted to immunoperoxidase staining using an avidin-biotin horseradish peroxidase technique.

32.97 How Sensitive Are the Tests?

Of the 50 specimens evaluated in one study, there was 94 % concordance between conventional Diff-Quik staining and immunoperoxidase staining. The organism is far easier to see with the immunoperoxidase stain [80]. Two Diff-Quik-positive specimens failed to stain with the immunoperoxidase method, and one Diff-Quik-negative specimen was detected by immunoperoxidase staining.

32.98 How Specific Are the Tests?

In a study of alkaline phosphatase anti alkaline phosphatase complex technique for the detection of *Pneumocystis carinii* in bronchoalveolar lavage fluid from 83 HIV-1 positive patients, 28 samples were positive by immunofluorescence, 26 by Grocott staining and 29 by immunohistochemistry [81].

32.99 Does Fixation Affect the Test?

Limited data are available.

Leshmaniasis

32.100 How Sensitive Are the Tests?

Limited data are available. Some data suggest a sensitivity of 90.9 % with PCR vs. 68.8 % with IHC [82]. PCR and ELISA assays are used more commonly [83, 84].

32.101 How Specific Are the Tests?

Some antibodies cross-react with fungi

32.102 Does Fixation Affect the Test?

Limited data are available

32.103 How Should I Incorporate These Tests into My Practice?

Many fungal organisms are quite easily seen in H&E sections, while others such as *Histoplasma capsulatum* may be more difficult to find. The BCG antibody is very useful as a screening method to detect a wide variety of pathogens, especially when pathological features suggest an infection, but no microorganism can be cultured or when only formalin-fixed tissue samples are available. Specific IHC testing for is not yet in widespread clinical use but has been developed and tested for a limited number of organisms. These tests are of greatest utility when rapid accurate specific identification is essential for therapeutic purposes.

Figures 32.16, 32.17, 32.18, 32.19, 32.20, 32.21, and 32.22

Table 32.4.

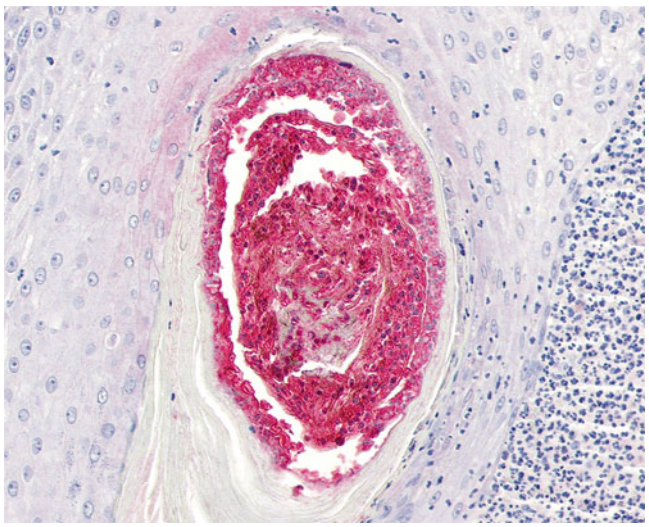

Fig. 32.16 Dermatophytosis anti-BCG IHC ×200

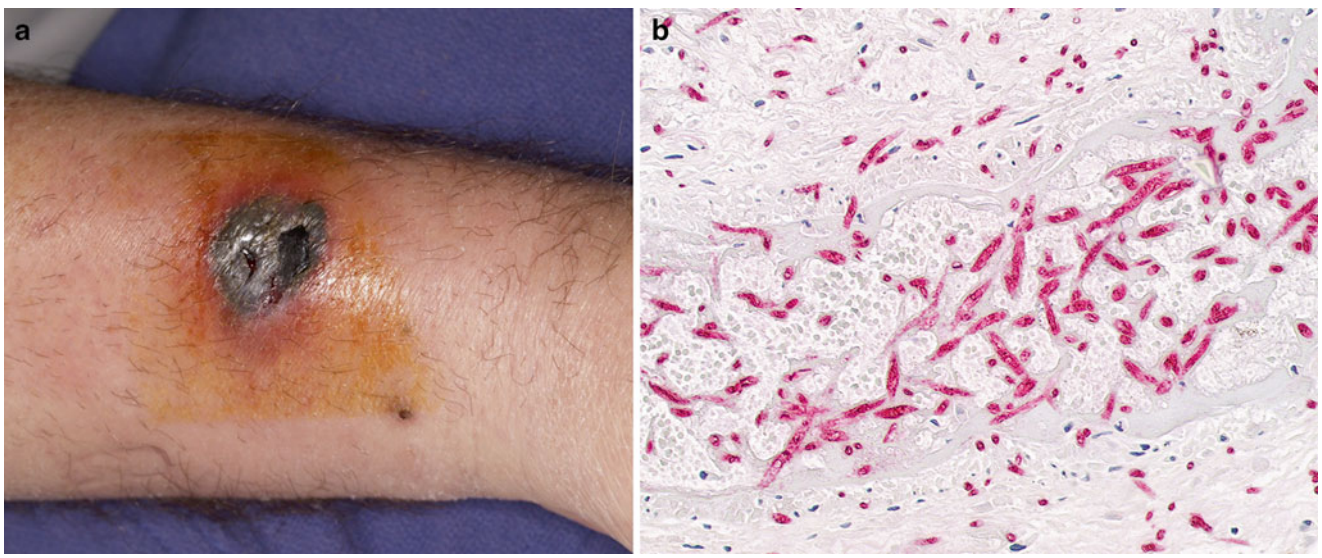

Fig. 32.17 (**a**) *Aspergillus* sepsis, (**b**) *Aspergillus* sepsis anti-BCG IHC ×400

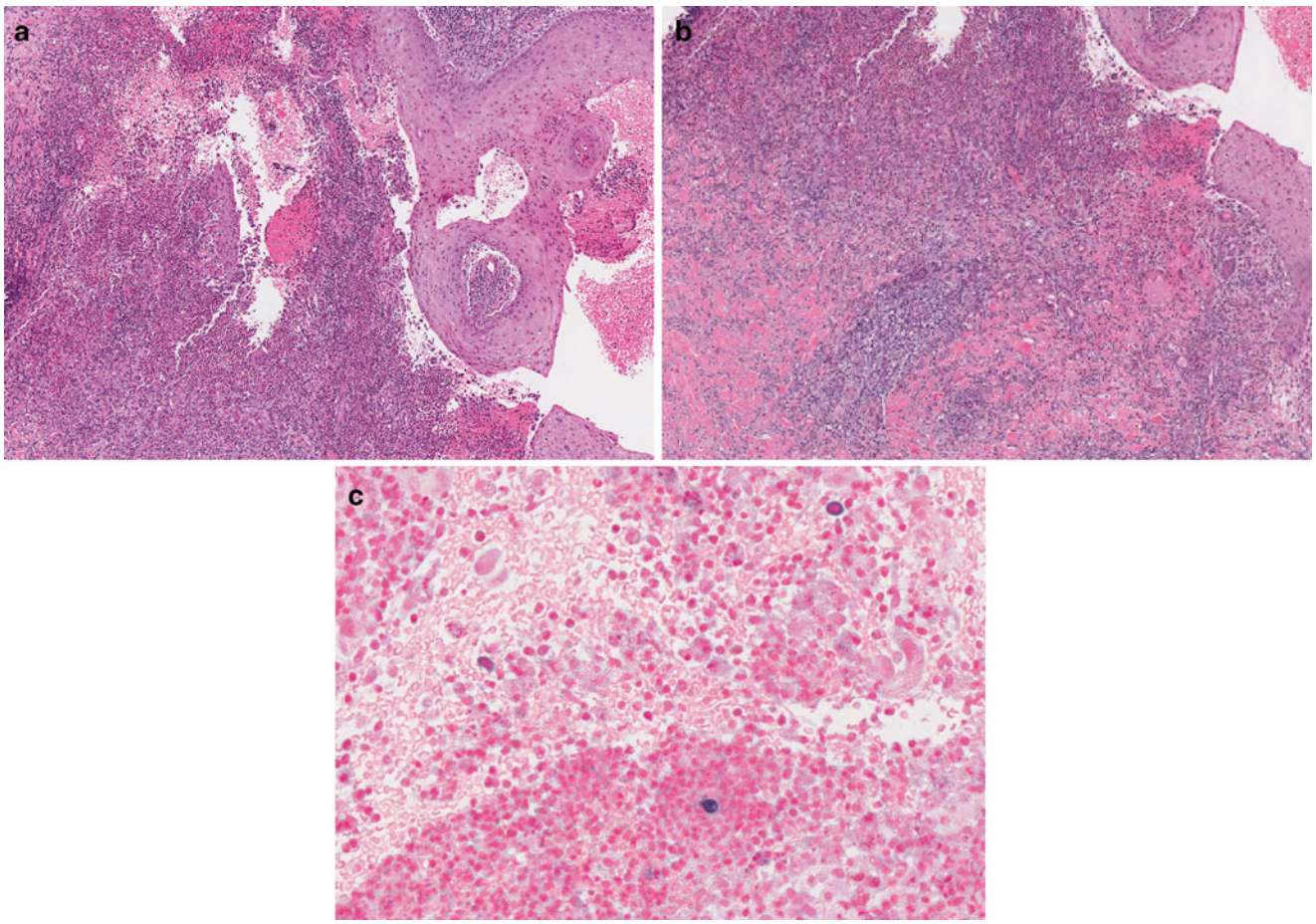

Fig. 32.18 (**a**) *Blastomyces* H and E ×40, (**b**) *Blastomyces* H and E ×60, (**c**) *Blastomyces* ISH ×120

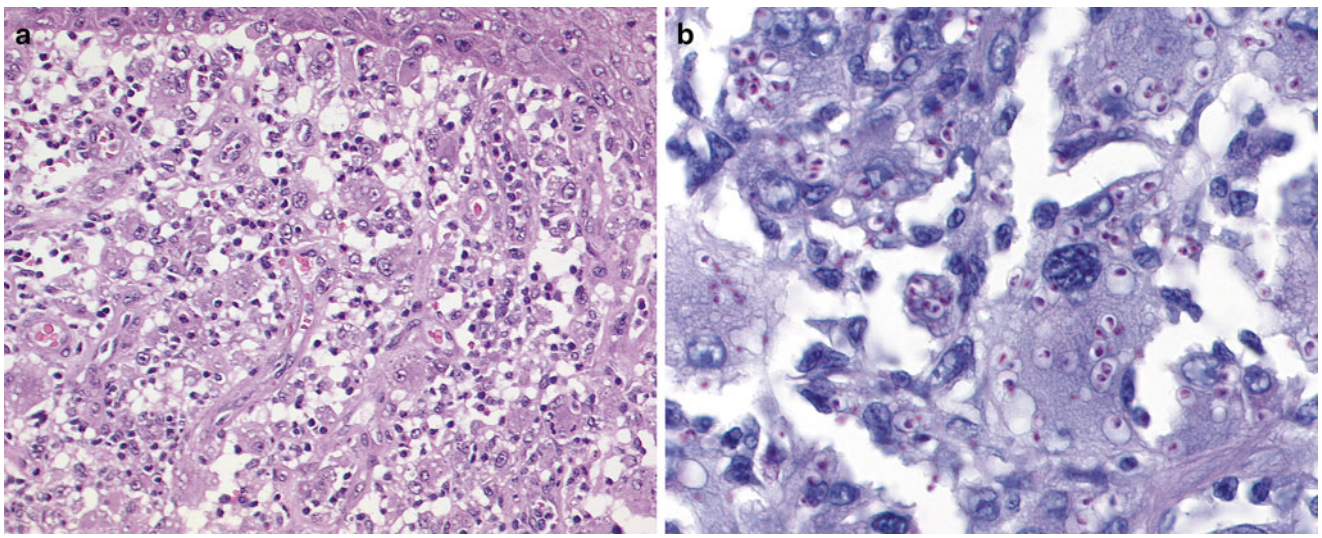

Fig. 32.19 (**a**) Histoplasmosis H&E ×400, (**b**) Histoplasmosis ISH ×600

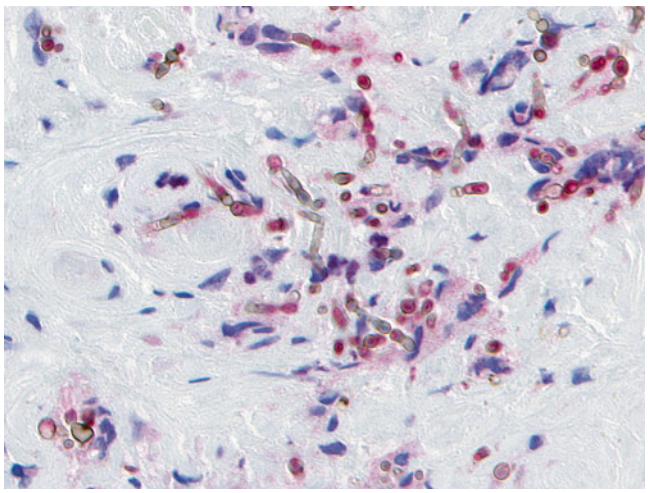

Fig. 32.20 Fungi cross-reacting with leishmanial antibody (polyclonal anti-Leishmania AB, Cristina Riera, M.D.) IHC ×400

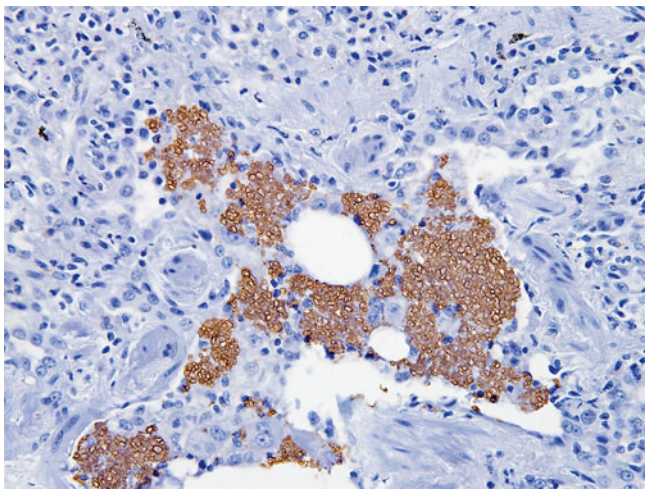

Fig. 32.21 *Pneumocystis carinii* IHC ×200

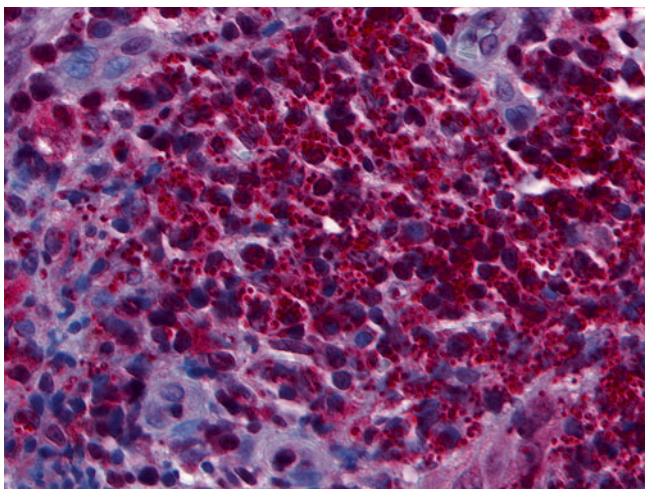

Fig. 32.22 Leishmaniasis (polyclonal anti-Leishmania AB, Cristina Riera, M.D.) IHC ×400

Table 32.4 Commonly used immunohistochemical stains for the detection of fungi

Stain	Comment
Anti-*BCG*	Useful as a screening method to detect a wide variety of pathogens, especially when pathological features suggest an infection, but no microorganism can be cultured or when only formalin-fixed tissue samples are available
Fungal-specific stains	Evolving technology. Cross reactions remain common. ISH is highly specific for a limited number of organisms

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