Application of *in situ* hybridization for the diagnosis of amoebic encephalitis

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Among free-living amoebae, *Naegleria fowler*i, *Balamuthia mandrillaris* and *Acanthamoeba* spp. are known causative agents of amoebic encephalitis in humans. Immunohistochemical methods using anti-amoeba antibodies have been used for diagnostic separation of these amoebal pathogens. However, anti-amoeba antibodies are limited in practical use because they are not available commercially and are not easy to develop in house. We have used *in situ* hybridization (ISH) for diagnosis of amoebic encephalitis on histological preparations of central nervous system tissue from mice experimentally infected with the amoebae. Oligonucleotide probes (approximately 40 bp) specific to each amoeba were designed based on the sequence of the 18S ribosomal RNA gene and were used for whole-cell hybridization. The probes, which contained a biotin at the 5'-end, were detected with streptavidin-conjugated HRP or Alexa 488. Specificity and reactivity of the probes were confirmed by staining the respective amoebae prior to histological application. High-resolution ISH images of the amoeba cells with excellent contrast were consistently obtained with fluorescence light microscopy. The advantage of ISH for the detection of specific pathogens in pathological specimens might be queried because of the complexity of the stain technology. However, probes specific to pathogenic microbes can be developed easily once the DNA sequence data are available.