

## **Risk Factors Associated with Plastination: II. Infectious Agent Considerations**

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*Abstract:* The technique of plastination often requires handling organs and tissues of human or animal origin. An ongoing concern associated with the plastination process has been risk of contracting infectious pathogens as the preparator works with such tissues. Human pathogens, including the agent causing Creutzfeldt-Jakob disease (CJD) or the human immunodeficiency virus (HIV), and animal pathogens that affect humans (e.g., rabies virus, *E. coli* bacteria) have been of particular concern. This paper provides an overview of several viral and bacterial pathogens that may be of concern to plastinators as well as recommended methodologies for avoiding infection by these agents.

*Key words:* **Creutzfeldt-Jakob; HIV; pathogen; plastination; risk**

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### **Introduction**

Individuals handling fresh human or animal tissues must continually be mindful of infectious agents that may be contained in these tissues. The risk of contracting disease from such tissue handling should be quite low with adequate safeguards. However, due to the potentially serious consequences of some of these diseases, potential risk cannot be overlooked. This paper is by no means an exhaustive review of infectious agent health risks associated with plastination. Instead, it is meant to provide a general overview of pathogens that may be encountered by individuals practicing plastination. This information has been available in the literature for some time and has not greatly changed in recent years. For the convenience of journal readers, we review bacterial and viral pathogens that may be of particular concern to preparators handling human or animal tissues. Concern regarding the Creutzfeldt-Jakob pathogen prion has increased in recent years, thus this agent is also discussed.

### *Infectious agent concerns related to plastination:*

A major concern of tissue handling associated with plastination is exposure of the preparator to pathogens. The risk of exposure to viable pathogens is greatest early in the preparation process, when tissues are still fresh and body fluids are still liquid. For low-risk as well as high-risk organisms in both human and veterinary fields, simple and common sense practices involving cadaver and tissue handling serve to greatly reduce exposure to, and therefore infection by, pathogens. Such procedures include: wearing of protective items such as gloves, surgical masks, eye protection and aprons whenever handling fresh tissue; avoidance of aerosolization of body fluids by careless handling or use of bone saws outside of hoods; taking care to avoid accidental cuts or needle sticks and paying prompt attention to such injuries when they do occur; careful cleaning and disinfection of surfaces and instruments; and proper disposal of unused tissues and

tissues intended for plastination.

One report described successful use of a formic acid-formalin technique for histological tissue preparation in both inactivating the infectious agent in CJD-infected mouse brains and preparing excellent-quality slides (Brown et al., 1990b). This treatment was described as effective in "almost completely eliminating infectivity in sections that were histologically indistinguishable from formalin-fixed material." This method was suggested as "virtually eliminating the risk of handling infectious material in neuropathologic processing of tissues from patients with CJD" (Brown et al., 1982). However, caution dictates careful consideration of the wording "almost completely." Further, note must be taken that this near-complete elimination of infectious agent took place while processing 4-5 mm slices of tissue, rather than large blocks of tissue or entire organs. The important question remains whether this fixation protocol would also be effective when processing larger blocks of tissue and/or entire organs, as is the normal situation in gross anatomical preparation. Indeed, Brown et al. (1990a) confine their remarks to fixation of histological materials. Prudence thus dictates that no cadaver of an individual diagnosed with CJD ever be used as a source of organs or large body parts for teaching in any mode. Further, the best degree of caution would be to avoid using tissues from any individual diagnosed with even non-specific neurologic signs.

It should be comforting that, for all of the above organisms except that causing CJD, handling the finished plastinated product would be expected to be associated with essentially no risk of disease transmission. This is true because the plastination process sequentially exposes tissues or organs to multiple procedures that independently or conjointly serve to render such pathogens non-infective.

## Literature cited

- Barkley WE. 1981: Containment and Disinfection. *In*: Kreig N, Briggs Phillips G, editors. *Manual of Methods for General Bacteriology*, American Society for Microbiology. Washington, DC: ASM Press.
- Bond WW, Favero MS, Petersen NJ, Ebert JW. 1983: Inactivation of hepatitis B virus by intermediate-to-high level disinfectant chemicals. *J Clin Microbiol* 18:535-538.
- Brown P, Gibbs CJ Jr, Amyx HL, Kingsbury DT, Rohwer RG, Sulima MP, Gajdusek DC. 1982: Chemical disinfection of Creutzfeldt-Jakob disease virus. *N Engl J Med* 306:1279-1282.
- Brown P, Gibbs CJ Jr, Gajdusek DC, Cathala F, LaBauge R. 1986: Transmission of Creutzfeldt-Jakob disease from formalin-fixed, paraffin-embedded human brain tissue. *N Engl J Med* 315:1614-1615.
- Brown P, Liberski PP, Wolff A, Gajdusek DC. 1990a: Resistance of scrapie infectivity to steam autoclaving after formaldehyde fixation and limited survival after ashing at 360°C: practical and theoretical implications. *J Infect Dis* 161:467-472.
- Brown P, Wolff A, Gajdusek C. 1990b: A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. *Neurology* 40:887-890.
- Centers for Disease Control. 1987: Recommendations for prevention of HIV transmission in health-care settings. *Morbidity and Mortality Weekly Report* 36 (Suppl. 3):S3-S18.
- Centers for Disease Control. 1989: Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. *Morbidity and Mortality Weekly Report* 38 (Suppl. 6):S1-S37.
- Favero MS, Bond WW. 1991: Chemical disinfection of medical and surgical materials. *In*: Block SS, editor. *Disinfection, Sterilization, and Preservation*, 4<sup>th</sup> ed. Philadelphia: Lea and Febiger. p 617-641.
- Gajdusek DC, Gibbs CJ, Collins G, Taub RD. 1976: Survival of Creutzfeldt-Jakob disease virus in formalin-fixed brain tissue. *N Engl J Med* 294:553.
- Gajdusek DC, Gibbs CJ Jr, Asher DM. 1977: Precautions in medical care of, and in handling materials from, patients with transmissible virus dementia (Creutzfeldt-Jakob disease). *N Engl J Med* 297:1253-1258.
- Handsfield HH, Cummings J, Swenson PD. 1987: Prevalence of antibody to human immunodeficiency virus and hepatitis B surface antigen in blood samples submitted to a hospital laboratory. *JAMA* 258:3395-3397.
- Hunter GD, Gibbons RA, Kimberlin RH, Millson GC. 1969: Further studies of the infectivity and stability of extracts and homogenates derived from scrapie affected mouse tissue. *J Comp Pathol* 79:101-108.
- Kingsbury DT, Amyx HL, Gibbs CJ Jr. 1983: Biophysical properties of the Creutzfeldt-Jakob agent. *In*: Court LA, editor. *Virus non-conventionnels et affections du système nerveux central*. Paris: Masson. p 125-137.
- Manuelidis EE, Angelo JN, Giorgacz EJ. 1977: Experimental Creutzfeldt-Jakob disease transmitted via the eye with infected corneas. *N Engl J Med* 296:1334-1336.
- Manuelidis EE, Kim JH, Mericangas JR, Manuelidis L. 1985: Transmission to animals of Creutzfeldt-Jakob disease from human blood. *Lancet* 2:896-897.
- Nicholson KG. 1987: Rabies. *In*: Zuckerman AJ, Banatvala JE, Pattison JR, editors. *Principles and Practice of Clinical Virology*. New York: John Wiley

contamination with chemical germicides of all used instruments and surfaces should follow all procedures. Of course, the waste generated during these procedures should be properly identified as biohazardous as well as properly disposed. While simple, these procedures are extremely effective for minimizing risk of exposure and infection by most human pathogens that may be encountered during the early stages of specimen preparation (Rutala, 1995).

Embalming fluids, which contain formalin, completely inactivate HIV (CDC, 1987). Thus, assuming complete fixation via good perfusion and/or adequate contact time, formalin fixation should render tissues safe. The HIV virus demonstrates no inordinate stability in the environment and is subject to rapid inactivation by drying in ambient air. Thus, extraordinary procedures are unnecessary for decontamination of potentially contaminated surfaces and instruments. Standard sterilization procedures are adequate for sterilizing instruments and surfaces contaminated with body fluids including blood from individuals infected with HIV (CDC, 1987). Sodium hypochlorite (household bleach) also effectively inactivates the HIV virus. The most important caveat here relates to the degree of contamination of the surface or instrument with organic material (since organic contamination renders hypochlorite less effective) (Rutala, 1995). Solutions between 500 and 5000 ppm (1:100 to 1:10 dilution) of household bleach are effective, depending on the degree of contamination of the object.

Hepatitis B virus is another pathogen of particular concern. Like HIV, exposure to infected blood is the single most important means of transmission of this disease (CDC, 1989). Sterilization/disinfection agents with activity ranked "intermediate to high" have been shown to be effective against HBV (Bond et al., 1983). Due to similarities in transmission and sensitivity to sterilization agents, adoption of the same barrier protection and sterilization procedures recommended for HIV will also minimize exposure to and infection by HBV. However, one critical difference between these two diseases is that an effective vaccine (90% effective for at least 7 years following immunization) for HBV is available. Indeed, the CDC as well as other groups recommends that individuals routinely exposed to human blood receive immunization for HBV. This extends beyond health care workers to include public safety personnel such as firefighters, law-enforcement and correctional-facility personnel (Handsfield et al., 1987; CDC, 1989). Individuals routinely involved in anatomical preparation of human tissues may of course also benefit from such protection.

Other human hepatitis viruses (non-A and non-B) are also currently of potential relevance to plastinators. Because these viruses are not reliably culturable, categorical statements as to the effectiveness of various disinfectants or sterilants are difficult to make. Nonetheless, the current consensus is that these viruses are not particularly resistant to disinfectants (Favero and Bond, 1991).

The single organism that departs significantly from the above generalizations is the agent causing Creutzfeldt-Jakob disease (CJD). This disease is caused by an unconventional virus-like organism that causes a spongiform encephalopathy. Though the incubation period may vary from months to many years, once clinical signs present, the disease is inevitably fatal. Neither treatment nor vaccine is available for this disease. Furthermore, CJD is uniquely resistant to nearly all routine sterilization procedures. Thus, CJD should be considered separately from all other pathogens.

Although the CJD agent resides mainly in and causes its main clinical signs related to tissues of the central nervous system (including the optic nerve and the cerebrospinal fluid), many other organs carry sufficient levels of pathogen to be infectious to humans (Rosenberg et al., 1985). Such tissues include liver, lung, lymph nodes, kidneys, corneas, white blood cells, whole blood and urine (Manuelidis et al., 1977; Gajdusek et al., 1977; Manuelidis et al., 1985; Tateishi, 1985). The CJD agent presents extreme resistance to denaturation or destruction. Indeed, transmission of the disease has been effected following exposure to formalin-fixed and paraffin-embedded human brain tissue routinely prepared for histopathological examination (Brown et al., 1986). The infectious agent has long been shown to be resistant to most fixatives, including formaldehyde (Zlotnik and Stamp, 1965; Gajdusek et al., 1976; Brown et al., 1982; Brown et al., 1986). Indeed, formalin fixation actually stabilizes the pathogen against autoclaving (Taylor and McConnell, 1988; Brown et al., 1990a). Routine disinfectants and sterilants as well as extreme heat are also ineffective against the agent. Procedures that are effective against the CJD agent include steam autoclaving at 132°C for one hour, immersion in 1N sodium hydroxide at room temperature for one hour (Rosenberg et al., 1985) and exposure to high levels of phenol (far exceeding those used in fixative solutions) (Hunter et al., 1969; Kingsbury et al., 1983). Exposure to a 5000 ppm hypochlorite has also been reported as effectively inactivating the pathogen (Brown et al., 1982). Although these techniques are effective for instruments and surfaces, they are incompatible with treatment of

tissues intended for plastination.

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## Literature cited

- Barkley WE. 1981: Containment and Disinfection. *In*: Kreig N, Briggs Phillips G, editors. *Manual of Methods for General Bacteriology*, American Society for Microbiology. Washington, DC: ASM Press.
- Bond WW, Favero MS, Petersen NJ, Ebert JW. 1983: Inactivation of hepatitis B virus by intermediate-to-high level disinfectant chemicals. *J Clin Microbiol* 18:535-538.
- Brown P, Gibbs CJ Jr, Amyx HL, Kingsbury DT, Rohwer RG, Sulima MP, Gajdusek DC. 1982: Chemical disinfection of Creutzfeldt-Jakob disease virus. *N Engl J Med* 306:1279-1282
- Brown P, Gibbs CJ Jr, Gajdusek DC, Cathala F, LaBauge R. 1986: Transmission of Creutzfeldt-Jakob disease from formalin-fixed, paraffin-embedded human brain tissue. *N Engl J Med* 315:1614-1615.
- Brown P, Liberski PP, Wolff A, Gajdusek DC. 1990a: Resistance of scrapie infectivity to steam autoclaving after formaldehyde fixation and limited survival after ashing at 360°C: practical and theoretical implications. *J Infect Dis* 161:467-472.
- Brown P, Wolff A, Gajdusek C. 1990b: A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. *Neurology* 40:887-890.
- Centers for Disease Control. 1987: Recommendations for prevention of HIV transmission in health-care settings. *Morbidity and Mortality Weekly Report* 36 (Suppl. 3):S3-S18.
- Centers for Disease Control. 1989: Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. *Morbidity and Mortality Weekly Report* 38 (Suppl. 6):S1-S37.
- Favero MS, Bond WW. 1991: Chemical disinfection of medical and surgical materials. *In*: Block SS, editor. *Disinfection, Sterilization, and Preservation*, 4<sup>th</sup> ed. Philadelphia: Lea and Febiger. p 617-641.
- Gajdusek DC, Gibbs CJ, Collins G, Taub RD. 1976: Survival of Creutzfeldt-Jakob disease virus in formalin-fixed brain tissue. *N Engl J Med* 294:553.
- Gajdusek DC, Gibbs CJ Jr, Asher DM. 1977: Precautions in medical care of, and in handling materials from, patients with transmissible virus dementia (Creutzfeldt-Jakob disease). *N Engl J Med* 297:1253-1258.
- Handsfield HH, Cummings J, Swenson PD. 1987: Prevalence of antibody to human immunodeficiency virus and hepatitis B surface antigen in blood samples submitted to a hospital laboratory. *JAMA* 258:3395-3397.
- Hunter GD, Gibbons RA, Kimberlin RH, Millson GC. 1969: Further studies of the infectivity and stability of extracts and homogenates derived from scrapie affected mouse tissue. *J Comp Pathol* 79:101-108.
- Kingsbury DT, Amyx HL, Gibbs CJ Jr. 1983: Biophysical properties of the Creutzfeldt-Jakob agent. *In*: Court LA, editor. *Virus non-conventionnels et affections du système nerveux central*. Paris: Masson. p 125-137.
- Manuelidis EE, Angelo JN, Giorgacz EJ. 1977: Experimental Creutzfeldt-Jakob disease transmitted via the eye with infected corneas. *N Engl J Med* 296:1334-1336.
- Manuelidis EE, Kim JH, Mericangas JR, Manuelidis L. 1985: Transmission to animals of Creutzfeldt-Jakob disease from human blood. *Lancet* 2:896-897.
- Nicholson KG. 1987: Rabies. *In*: Zuckerman AJ, Banatvala JE, Pattison JR, editors. *Principles and Practice of Clinical Virology*. New York: John Wiley

& Sons.

Rosenberg RN, White III CL, Brown P, Gajdusek C, Volpe JJ, Posner J, Dyck J. 1985: Precautions in handling tissues, fluids, and other contaminated materials from patients with documented or suspected Creutzfeldt-Jakob disease. *Ann Neurol* 19:75-77.

Rutala WA. 1995: Antisepsis, disinfection, and sterilization in hospitals and related institutions. *In*: Murray, PR, editor. *Manual of Clinical Microbiology*, 6<sup>th</sup> ed. Washington DC: ASM Press, p 227-245.

Tateishi J. 1985: Transmission of Creutzfeldt-Jakob disease from human blood and urine into mice. *Lancet* 2:1074.

Taylor DM, McConnel I. 1988: Autoclaving does not decontaminate formol-fixed scrapie tissues. *Lancet* 1:1463-1464.

Zlotnik I, Stamp JT. 1965: Scrapie in a Dorset Down ram. A confirmation of the histological diagnosis by means of intracerebral inoculation of mice with formol fixed brain tissue. *Vet Rec* 77:1178-1179.

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