

Infectious risks in autopsy practice

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Abstract: The purpose of this article is to analyze whether after proper decontamination using standardized methods there are still infectious markers in the autopsy room, suggestive for the possibility of contacting infectious diseases from previous autopsies. In order to analyze the presence and severity of residual risk we performed the following procedures: air sampling for bacterial agents, microbiological identification on surfaces and immunoassays for viruses/agents with slow growth/no growth on culture mediums. After autopsy room disinfection we found an increased number of Gram positive cocci (especially enteric), a decreased number of Gram negative bacteria and two positive immune reactions – one for HVB and one for HIV. As a conclusion, standard prophylactic procedures must always be used, as autopsy-related infectious risk is significantly increased compared to other specialties. Also, standard disinfection has a limited value in removing viral traces, leading to a residual infectious risk.

Key Words: infectious risks, autopsy infection, HIV, hepatitis

Medical-legal practice is associated with a significantly increased risk compared to other medical specialties, both in terms of airborne diseases and diseases transmitted parenterally; various studies revealed increased prevalence of HIV, hepatitis B, C, D, G, tuberculosis, prion diseases, hantavirus, measles, HTLV-1 or bacterial infections in autopsy workers. [1-8]. The purpose of this article is to analyze whether after proper decontamination using standardized methods there are still infectious markers in the autopsy room, suggestive for the possibility of contacting infectious diseases from previous autopsies.

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Material and methods

For identifying the infectious risk the following procedures were performed:

1. Air sampling in the autopsy room. Microbial detection the air was performed using Petri dishes containing agar, blood-agar, Chapman, bile-esculine-agar and MacConkey mediums (see Table 1). The Petri dishes were left open for 10 minutes in various locations in the autopsy room (three on autopsy tables, two near the windows, one near the main door); testing was performed at 7.00 and 7.10 AM in two non-consecutive days. After 10 minutes the dishes were closed and put in a thermostat for 24 hours at 37 degrees Celsius.

Table 1. Culture mediums and identifiable species

Medium	Identifies
Agar	E.coli, Ps.aeruginosa, St.aureus, Str. pyogenes
Bile-Esculin-Agar	Enterococcus faecalis, St.faecium, Yersinia enterocolitica, E.coli
McConkey	Enterobacter aerogenes, E.coli, S. cholerasuis, St.aureus
Chapman	St.aureus, St.epidermidis, S.coli
Agar-Blood	Nisseria (m,g), Str.pneumonie, St.aureus, Str.pyogenes

2. Identification of bacterial agents on surfaces. For this purpose samples were taken from the autopsy room using cotton swabs soaked in sterile tubes containing 1 ml saline. Samples were taken from the main door, three autopsy tables, two windows, in two non-consecutive days, between 7.15 and 7.30 AM. The solute was concentrated by centrifugation and then Gram slides were prepared using the standard technique.

3. Immunoassays were used for the identification of infectious agents with high risk of transmission and with slow growth/no growth on standard culture mediums, or viral agents. Testing was conducted using ELISA assays for AgHBs, AgHAV (IgG, IgM), Ac anti HCV, Ag HIV1 si HIV2, flu A/B antigens, Ac anti Toxoplasma (IgG, IgM), Ac anti BK (by Standardia) and multi HBs (Ag HBs, HBc, Hbe, Ac anti HBs, HBc, HBe) (by Biotech). Samples were taken from autopsy rooms using cotton swabs soaked in sterile tubes containing 1 ml saline, from the main door, three autopsy tables, two windows, in two non-consecutive days around 7.30 AM.

Statistical analysis was conducted using the SPSS v19 software, by using the following methods: descriptive (mean, median, frequencies), and correlations (Pearson). A p value of 0.05 was considered significant whilst a value below 0.01 was considered highly significant.

Results

Air sampling in the autopsy room

Air sampling was conducted using five culture mediums (agar, agar-blood, Chapman, MacConkey, BEA). The mean values obtained at 10 minutes were normalized and a total number of CFU/m²/h was computed by multiplying the values previously obtained with 11.1 (a Petri dish was a surface of 9 cm²).

Table 1. Culture mediums and identifiable species

Medium	N CFU/10min *)	Normalization**)	CFU/m ² /h
Agar	7	42	466
Agar-blood	8	48	533
Chapman	2	12	155
MacConkey	<1	Nd	Nd
BEA	8	48	533

*) the value was obtained by dividing the sum of all CFUs on a certain medium with the number of plates; values were rounded to the closest integer.

**) the value previously obtained was multiplied by six, in order to obtain a medium number of CFUs per hour

positive cocci, sometimes in diplo or small strings;

Identifying surface flora

As our previous procedure revealed a significantly decreased number of Gram-negative strains in the air samples we tried to verify whether this result is similar to the one on autopsy room surfaces. The Gram stains we used has identified mostly Gram

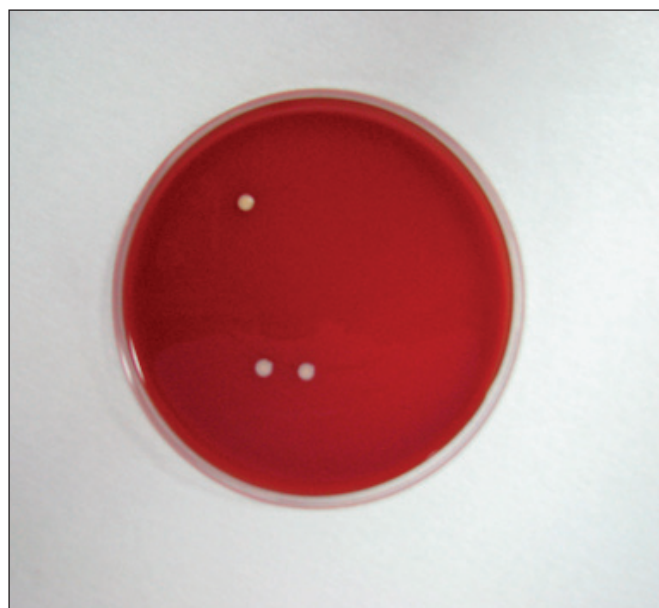


Figure 1. Blood-agar medium culture with smooth, creamy yellowish colonies of about 3 mm

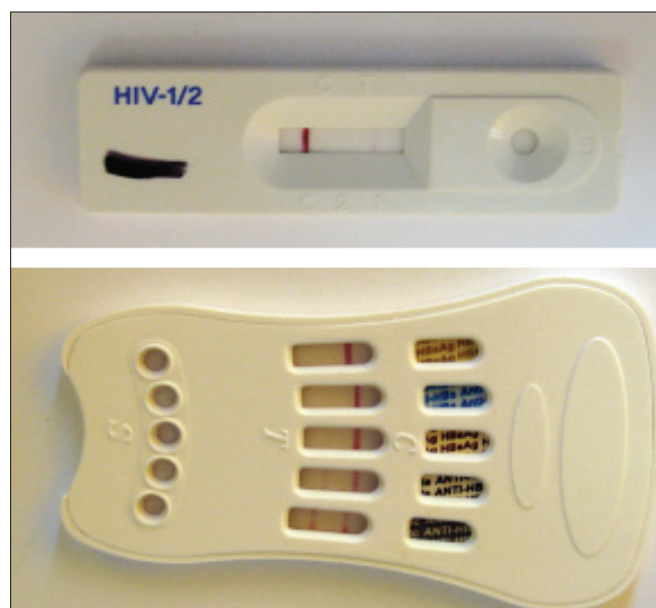


Figure 2. Positive ELISA reactions for HIV1 (up) and HVB (bottom)

Gram positive bacilli were only rarely identified, with a diptheroid morphology and only very rarely we could identify Gram negative bacilli. The results are therefore similar to those obtained by air sampling.

Immunoassays

ELISA assays were used to test for other infectious agents, not identifiable using the methods previously used. We analyzed the presence of viral hepatitis (A, B, C – the most frequent in Romania), HIV, Toxoplasma, orthomixoviridae and TBC. Positive results were only obtained for Hepatitis B (using a multiassay kit) and HIV. The epidemiological survey revealed a positive HIV autopsy 24 hours before sampling on the autopsy room from which the sample was obtained, and gave negative results for the HVB case. We must again mention that testing were conducted randomly, almost 24 hours after autopsies; therefore we measured only the residual effect, caused by deficient disinfection.

Discussions

Infectious risk associated with autopsy activity was not analyzed in the last years in Romania. Studies conducted in other countries revealed a significantly increased risk especially for tuberculosis and hepatitis B.

Agar and blood-agar gave close values (a mean of 466 and 533 CFU/m²/h respectively), values which could not be correlated with the position in the autopsy room. Chapman is known to better identify pathogen *Staphylococcus* species, as it contains a high concentration of salts. Mean number of CFU's is significantly decreased compared to gelose and gelose-agar; however, the values obtained (associated with the fact that the colonies had a typical appearance – smooth, creamy, 2-3 mm) suggests a possible risk for transcutaneous staphylococcal infections in the autopsy room if a person enters in here with open wounds. BEA is a selective medium for identifying Group D *Streptococcus* species; it has the advantage of differentiating Group D from other *Streptococcus* species by their unique properties of being able to grow on a medium containing 4% bile and of metabolizing esculine to glucose and esculetine.

Group D *Streptococci* are present in the digestive and urinary systems; therefore when opening them during the autopsy the contamination is very high. Even is they are associated with various infectious diseases in humans, their presence in the air does not seem to be dangerous as no study was able determine a correlation between air-borne enterococci and human diseases. Precautions should however be taken by immune-depressed persons, as their numbers seem very

high in the autopsy room (533 CFU/m²/h). MacConkey medium contains bile salts (inhibitory for most Gram positive strains except for Group D Streptococci), cresyl violet (inhibitory for most Gram positive strains), lactose, peptone and neutral red.

In our study the number of CFUs grown on this medium was extremely low (with a mean below 155 CFU), an apparently paradoxical situation as the number of bacteria grown on BEA was very high (usually normal digestive flora contains a higher percentage of Gram negative bacteria than enterococci). Possible causes may be a diminished survival outside the body for Gram negative strains or an increased removal rate for Gram negatives by our usual disinfection methods. The maximal number of CFUs/m²/h obtained for blood-agar is within acceptable limits for a general purpose hospital (see [9] for details); the standard procedure we used was slightly different - we used multiple samplings, multiple culture medium, and sampling height was variable (however the mean was close to 1 meter). The values we obtained in different locations were not statistically significant therefore we could imply that the results are comparable. Also, these values were obtained in the morning, before autopsy practice, after standard disinfection. Therefore the values are probably much higher during or after autopsy hours.

In Romania the prevalence of tuberculosis in the general population is around 25/100.000 inhabitants. A study conducted in Iasi on a 32 years period regarding the prevalence of tuberculosis in physicians/nurses find the following: at a median annual number of 220 healthcare workers were identified a number of 60 cases of tuberculosis, mostly affecting nurses, followed by auxiliary personnel and physicians (six cases); mean annual rate was 1363/100.000, values ten times higher than the prevalence in the general population [10]. Autopsy personnel has a ten-fold increase in risk and the relative risk, compared to the general population [11], is 100-200 times greater. By extrapolating these information we can hypothesize that approximately one autopsy personnel out of five will have at least one active tuberculosis infection during his/hers lifetime. Mean infecting dose for BK is extremely low ($ID_{50} < 10$ bacilli) [12]. Our study did not find BK antigens after disinfection; this however does not exclude infection if positive cases are on the autopsy table.

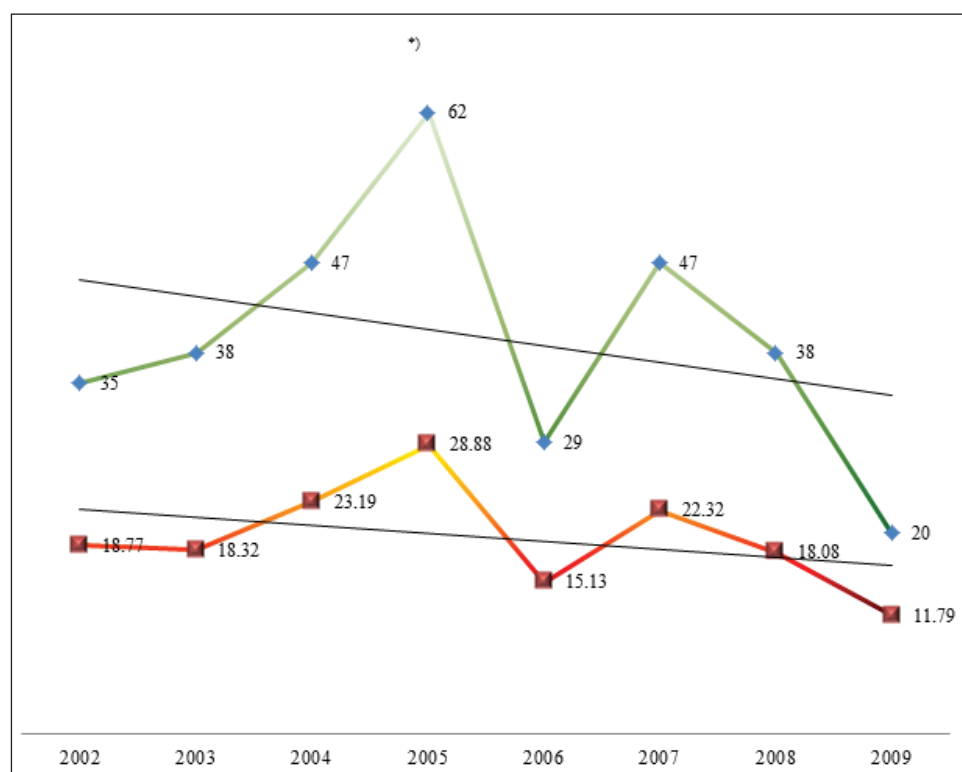


Figure 3. The number of cases with tuberculosis identified at the NILM between 2002 and 2009. We only took into consideration cases in which tuberculosis was macroscopically identified and was considered as a cause of death.

*)Values were multiplied by 10 for a better graphical analysis. (line above – number of cases, line below – percent multiplied by ten).

The risk of developing tuberculosis in forensic practice in Romania is mostly determined by (1) a very high number of autopsies on cadavers with macroscopic lesions and (2) insufficient safety measures – not using at least N95 masks as a minimal precaution. In the graph below we present the current situation with tuberculosis cases identified during the autopsy and which were considered to be the cause of death.

Out of 15935 autopsies conducted at the National Institute of Legal Medicine between 2002 and 2009 a total number of 316 positive cases were found (1.95%). If we take

this number and multiply it with ten (a minimal number of persons present in the autopsy room during one examination) we obtain a number of above 3000 potentially infecting contacts which, which, associated with an extremely low infecting dose (<10 bacilli) reveals the magnitude of this problem.

Hepatitis B has the highest transmissibility rate amongst all parenteral viruses, with a rate about 100 times greater than HIV; it can lead to a latent infection, with an increased risk of chronicity and hepatocellular carcinoma or an acute infection, with a high change of complete recovery. The contamination risk for health personnel is higher than in the general population, the highest risk being amongst those who work with blood or perform invasive procedures. For example in India physicians with more than 20 years field work have a prevalence of 30% whilst the prevalence in general population is 5%. CDC found that the general infecting risk in autopsy personnel is about 5% whilst if the blood contained contaminated with Ag HBe antigens, the value increased to about 30%[12]. As is the case with BK infection, the highest risk is amongst nurses/auxiliary personnel [1, 13]; for example in Austria there were identified the following relative risks: nurses – 30.6%, auxiliary personnel - 30.4%, physicians – 13.9%, laboratory personnel 2.9% and other 22.3%[14].

Studies conducted in US found the highest risk for HVB infection was amongst surgeons and pathologists (about 6%)[15]. HVB is present in all body fluids, including saliva, blood, semen, CSF. The infection usually occurs parentally (needle stick), but also by exposing mucosal tissue to the infecting fluid (or fluid particles, developed inclusive during opening the cranial cavity, which can easily reach the conjunctiva or the oral cavity). Unlike HIV, HVB is able to survive outside the body for seven days in dried blood spots or other dried body fluids. Minimal infecting dose depends upon the genotype of the infective strain; for example in chimpanzees DM_{50} is about 10 copies for genotype A and 50 copies for genotype C[16]. One ml of infective blood contains 10^2 - 10^9 viral copies; therefore even a microscopically blood particle can contain an infecting dose. Our study found one positive reaction for HVB; the epidemiological survey could not identify a positive case in the last week suggesting that the positive status of the cadaver was not known during the autopsy, nor was suspected.

HIV infections were rarely cited – for example in 1992 a pathologist, while doing the autopsy of a person with progressive neurological decay cut himself; the cut was about 1 cm and was done while dissecting the scalp. An initial ELISA HIV testing was negative; however, a follow up test conducted after six weeks found a positive reaction.

In the USA, on an eleven year period (1991-2001) were identified a total number of 23951 cases of HIV infection in medical personnel; however 91% were not associated with professional exposure whilst in the other 9% a positive correlation between medical activity and the seropositivity was not established with certainty. In only about 200 cases a positive correlation was established, mostly being determined by percutaneous exposures; in eight cases however the cause was cutaneo-mucosal exposure. For example a physician was tempting hemostasis for a seropositive patient without wearing surgical gloves; because of the increased blood pressure the hemostasis could not be properly performed subsequently leading to exposing his eyes, nose and mouth to the contaminated blood [17]. Our study found one positive HIV reactions, which was associated with a seropositive autopsy conducted on that table the day before.

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References

1. Aydin B, Tanyel E, Colak B, Fisgin N, Tulek N. Infection control implementations at forensic medicine practice: A national survey in Turkey. American Journal of Forensic Medicine and Pathology. 2009;30: 167-70.
2. Claydon SM. The high risk autopsy: Recognition and protection. American Journal of Forensic Medicine and Pathology. 1993;14: 253-6.
3. Morgello S, Mahboob R, Yakoushina T, Khan S, Hague K. Autopsy findings in a human immunodeficiency virus-infected population over 2 decades: Influences of gender, ethnicity, risk factors, and time. Archives of Pathology and Laboratory Medicine. 2002;126: 182-90.

4. Nolte KB, Taylor DG, Richmond JY. Biosafety considerations for autopsy. *American Journal of Forensic Medicine and Pathology*. 2002;23: 107-22.
5. Sanaei-Zadeh H, Amoei M, Taghaddosinejad F. Seroprevalence of HIV, HBV and HCV in forensic autopsies, of presumed low risk, in Tehran, the capital of Iran. *Journal of Clinical Forensic Medicine*. 2002;9: 179-81.
6. Templeton GL, Illing LA, Young L, Cave MD, Stead WW, Bates JH. The risk for transmission of *Mycobacterium tuberculosis* at the bedside and during autopsy. *Annals of Internal Medicine*. 1995;122: 922-5.
7. Touche S, Bajolet O. Laboratory infectious risk. *Risques infectieux au laboratoire*. 2010;2010: 65-70.
8. Trombert V, Huber P, Michel JP. Autopsy: death in the service of life. *Autopsie ou la mort au service de la vie*. 2005;20: 77-80.
9. Fisher G, Fodré S, Nehéz M. Das Ergebnis der Untersuchungen zur Feststellungen von Gesamtkeimzahl-Grenzwerten in der Luft von Operationsräumen. *Z Ges Hyg*. 1972;18: 729 - 33.
10. High risk of tuberculosis in health care workers in Romania. *The International Journal of Tuberculosis and Lung Disease*. 2008;12: 606-11.
11. Lundgren R, Norrman E, Asberg I. Tuberculosis infection transmitted at autopsy. *Tubercle*. 1987;68: 147-50.
12. CDC. Biosafety in Microbiological and Biomedical Laboratories 5th Edition: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, HHS Publication No. (CDC) 21-1112, Revised December 2009.
13. Curca C. Occupational bio-hazards in forensic medicine. *Din nou despre riscurile ocupationale in medicina-legala*. 1998;6: 91-5.
14. Maruna H, Westphal G. Epidemiology and risk calculation of hepatitis-B as an occupational disease in the Austrian health service. *International Archives of Occupational and Environmental Health*. 1986;57: 259-65.
15. West DJ. The risk of hepatitis B infection among health professionals in the United States: A review. *American Journal of the Medical Sciences*. 1984;287: 26-33.
16. Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T, et al. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C. *Transfusion*. 2008;48: 286-94.
17. Do ANMD, Ciesielski CAMD, Metler RPJD, Hammett TAMPH, Li JDPE, Fleming PLP. Occupationally Acquired Human Immunodeficiency Virus (HIV) Infection: National Case Surveillance Data During 20 Years of the HIV Epidemic in the United States, *Infection Control and Hospital Epidemiology*. 2003;24: 86-96.