Potential for Laboratory Exposures to Biohazardous Agents Found in Blood

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Abstract: The magnitude of risk for occupational exposures to biohazardous agents found in blood was assessed by 800 environmental samples taken from a total of 10 clinical and research laboratories at the National Institutes of Health (NIH). Thirty-one samples from 11 work stations in three laboratories contained hepatitis B virus surface antigen (HBsAg). Observations of workers indicated that environmental contamination arose from several sources. Among the 11 work stations with HBsAg environmental samples, eight had high work loads, seven had inappropriate behaviors, and nine had flawed laboratory techniques. This information suggests that a multifactorial approach is needed to minimize the risk of laboratory-associated infections. (*Am J Public Health* 1990;80:423–427.)

Introduction

Hepatitis B virus (HBV) infection is among the most commonly reported laboratory-associated infections.[1–4] Laboratory workers in an urban medical center are at almost three times the risk of acquiring HBV infection than other hospital employees, based solely on exposure to patients' blood.[5] HBV infection is seven to 10 times more common among occupational groups in laboratory medicine than in the general public.[6,7] Workers' anxiety is heightened because of the concomitant risk of acquiring other infections with human immunodeficiency virus (HIV) and agent(s) of non-A, non-B hepatitis.

Strict adherence to biosafety guidelines minimizes the risk of acquiring laboratory-associated infections.[8,9] Immunization of workers is clearly the most effective means to control infection.[10] However, many employees are reluctant to be immunized and, as is the case with HIV, licensed vaccines are not always available. Thus, these types of laboratory-associated infections continue to occur,[11] despite the fact that most are preventable. Recently, universal blood and body-fluid precautions were recommended to further enhance workers' safety.[12]

This study dealt with an assessment of similarities and differences in clinical and research laboratory activities concurrent with a survey to detect hepatitis B surface antigen (HBsAg) in the environment. For this study, HBsAg served as a marker for contamination with pathogens found in human blood. Based on data from viral survivability on fomites, the selection of HBsAg as a marker for blood-borne contamination exaggerates the viability of a labile microorganism such as HBV. Nevertheless, contamination with HBsAg suggests a common source and depicts a potential for transmission of infection in the laboratory. Given this caveat, the purpose of this study was to elucidate factors associated with HBsAg in the environment and, therefore, potential exposures to blood-borne pathogens in biomedical laboratories at the authors' institution.

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Methods

Recruitment and Stratification of Laboratories

Ten laboratories, representing a cross section of operations involving the use of human blood at the National Institutes of Health (NIH), were entered into a survey conducted from August 1984 through February 1985. We invited 119 laboratory workers to participate. Each volunteer was assigned a code to assure anonymity.

The laboratories surveyed fulfilled criteria for assignment to Biosafety Level II[8] and were chosen to represent a spectrum from high to low risk of workers handling blood containing HBV. The 10 participating laboratories were divided into 44 distinct work stations for purposes of data collection. Each of these stations was assigned a level of risk prior to environmental sampling based on the volume of blood processed and the hypothesis that the probability of detection would be greater in laboratories which processed blood drawn from individuals known to be infected with HBV than from those which excluded such blood samples. High-risk work stations were arbitrarily defined as those locations where the estimated volume of blood processed per week equaled or exceeded 1.5 L or the specimens received were from individuals known to belong to groups with a high prevalence of HBV infection. Stations assigned to the low-risk category were arbitrarily defined as areas where workers processed less than 1.5 L blood per week, with all blood drawn from donors known to be negative for HBsAg.

There were 75 hours of direct observation with the amount of time per laboratory depending on the continuity of tasks involving the use of blood. Observations were obtrusive because this approach allowed for interviews with workers while laboratory activities were assessed.[13] Data obtained in this manner may have been biased because of the worker's apprehension and/or attempts to modify unsafe practices. To minimize worker's reaction to being observed, the investigator sought to establish a good rapport with participants, reiterate key issues of the study, and assure the worker's anonymity. Stratification of risk in the work stations of the laboratories surveyed and the observation periods completed are listed on Table 1.

Laboratories 2 and 3 (Clinical Pathology and Transfusion Medicine Departments, respectively) provided diagnostic services for the Warren G. Magnuson Clinical Center (the hospital at NIH); the majority of work stations in these two laboratories were classified in the high-risk category. The work completed in Laboratory 4 was classified in the high-risk category because numerous specimens were analyzed for large-scale serological surveillance studies. Individuals in

TABLE 1—Distribution of Laboratories, Type of Operations, Level of Work Station Risk, and Observations

Laboratory		Categories of Risk		Observations (h)[b]
Code	Operation	High (No.)[a]	Low (No.)[a]	
1	Immunological profiles	0	2	2
2	Diagnostic	14	1	28
3	Diagnostic	11	1	15
4	Immunoassays	2	0	4
5	Immunological profiles	3	0	5
6	Immunological profiles	0	1	2
7	Flow cytometry	1	0	1
8	Liver disease research	3	0	4
9	Tumor immunology	3	0	9
10	Virology research	2	0	5
Total		39	5	75

[a]Number of work stations.
[b]Hours.

Laboratories 5, 7, 8, and 10 processed blood specimens that were obtained from patients with chronic liver disease or HIV infection. In Laboratory 9, a tumor immunology unit, workers analyzed specimens from patients likely to have a history of multiple blood transfusions.

Assessment of Laboratory Operations

Methods for assessing skilled performance (both behaviors and techniques) were adapted from a task strategies approach.[14] To control for spurious conclusions due to inconsistencies with data collection,[13,14] all data were recorded by a single investigator who reviewed the study parameters frequently to control for observer drift. No intervention was attempted during the study.

Observation of activities involving the use of human blood were assessed by two indices. First, the frequencies of 13 different manipulations were each tallied to estimate the extent of workers handling blood that was required in order to complete a task. A task was defined as the cumulative manipulations generating a single result. The second index was the work load (the number of blood specimens processed per week). These indices allowed for comparison of laboratories performing quite different operations.

Behaviors were also assessed in an attempt to identify practices associated with an increased risk of contamination. Specific behaviors were assessed when a worker performed a movement either accidentally or intentionally that involved contact between human blood and the employee's skin, conjunctiva, or mouth. Each behavior was classified either as appropriate, if the practice was consistent with biosafety recommendations,[8,9] or inappropriate, if the worker was placed at risk of an exposure (e.g., mouth pipetting fluids or skin contact with blood) as a result of a breach in good laboratory practices. In the event of an overt accident the worker's behavior was judged to be appropriate only if the contamination occurred despite good laboratory practices (e.g., mechanical failure or poor instrument design). Observations were tallied prior to knowledge of sample testing.

Environmental Survey

Samples collected for the detection of HBsAg were processed in a manner consistent with Biosafety Level II guidelines.[8] HBsAg was eluted from various surfaces using a modification of a procedure described by Favero, *et al.*[15,16] The elution fluid (FBS/PBS) was prepared using 20 percent fetal bovine serum (Lot #14-501B: M. A. Bioproducts, Walkersville, MD) (FBS), phosphate-buffered saline (0.15 M NaCl and phosphate: M. A. Bioproducts, Walkersville, MD) (pH 7.4 ± 0.5) (PBS), and sodium azide (1.0 mg/ml) (Sigma Chemical, St. Louis, MO).

Samples were collected from work surfaces measuring approximately 35 cm^2 using moistened swabs and from swatches of gloves worn by workers or absorbent paper placed on the work surface prior to handling blood. Pledgets (each with a surface area measuring 5.0 cm × 7.5 cm) cut from Grade 304 stainless steel by the Biomedical Engineering and Instrumentation Branch, NIH, Bethesda, MD, were used for indirect sampling. These pledgets were placed in the laboratories either in the working area or, for negative controls, outside the working space. Negative controls were graded unsatisfactory if the pledgets were either moved or visibly soiled during the exposure period. Metal pledgets were used because preliminary modeling studies demonstrated that nanogram amounts of HBsAg could be eluted from four surfaces (absorbent paper, glass, polystyrene, and stainless steel) with recovery rates averaging 20 percent, 77 percent, 82 percent, and 96 percent, respectively. Other data have shown that HBsAg can be recovered from metal surfaces with greater than 99 percent efficiency.[15] All samples were eluted in 1.5 ml FBS/PBS and stored at 4°C.

Hepatitis B Surface Antigen Detection

Samples were screened for the presence of HBsAg using a radioimmunoassay (Ausria II: Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions with these modifications: a) each control and sample was assayed in duplicate using 200 ul eluate per well; b) six distinct negative environmental controls were assayed with each run; and c) each assay tube was counted for 10 min. Tubes were counted in a gamma spectrophotometer (Tracor Analytic, Model 1185: Nuclear-Chicago, Des Plains, IL) with a calculated counting efficiency for I-125 of 83 percent. The average cpm for each paired assay was calculated. Samples with discrepant paired results were retested. Those samples with repeatedly discrepant results were excluded from the study.

A sample was classified as reactive if the cpm for the sample was greater than three standard deviations from the mean cpm for the negative controls. The coefficient of variation (CV) was calculated and the mean of the negative controls for each screening run (values ranged from 3 percent to 17 percent with a mean of 7 percent).

Reactive samples were confirmed using specific neutralizing antibody for HBsAg (anti-HBsAg) (Ausria II Confirmatory Neutralizing Kit: Abbott Laboratories, Chicago, IL). Appendix A outlines the method used for the confirmation assay. A sample was confirmed HBsAg positive if the reduction in the cpm of the sample reacted with anti-HBsAg was greater than 50 percent compared with the sample reacted with FBS/PBS and if these latter cpm were between the range of 50 percent to 150 percent of the mean cpm of the negative control. Reactive samples with cpm less than 1.5 times the mean cpm of the negative control were diluted beyond the sensitivity of this latter assay and were interpreted as borderline. Within run variation for the confirmation runs was expressed as the CV calculated for the negative control (values ranged from 39 percent to 59 percent with a mean of 4 percent).

Statistics

The association of the potential factors with HBsAg was assessed by determining odds ratios and 95 percent confidence intervals.[17]

Results

The results for the HBsAg sampling data are summarized on Table 2. These data show that our hypothesis was correct: high-risk operations do differ from low-risk operations in terms of the potential for blood-borne contamination in the laboratory. All environmental samples collected from the low-risk work stations were negative for HBsAg. A total of 69 samples collected from high-risk work stations in Laboratories 2–4 and 8–10 were reactive for HBsAg on the initial screening. Except for the tumor immunology research unit, these laboratories routinely processed blood drawn from individuals chronically infected with blood-borne pathogens. Approximately 55 percent (38/69) of the reactive samples were graded as borderline (low cpm for positive samples overlapped with high cpm for negative controls). Insufficient eluate volumes prohibited further analysis of these borderline samples. Thus, we analyzed a total of 800 samples, of which 31 (3.9 percent) were HBsAg positive, 38 (4.8 percent) were borderline, and 731 (91.4 percent) were negative.

Samples confirmed positive for HBsAg were collected from Laboratories 2, 3, and 8. The HBsAg positivity from these laboratories ranged from a high of 17.8 percent (8/45) (liver disease research unit) to a low of 1.4 percent (2/139) (Transfusion Medicine). The frequency of obtaining positive samples in Clinical Pathology was 8.6 percent (21/245).

Factors Associated with HBsAg in High-Risk Work Stations

Individuals who worked in nine different locations in the Clinical Chemistry and Hematology Services had the highest work loads (average 1,430 specimens/week: range from 210–2,700). Workers in the Transfusion Medicine Department averaged 106 specimens per week. The liver disease unit, the laboratory with the greatest percent of samples positive for HBsAg, averaged 27 specimens per week. Work loads for Laboratories 4 and 7 averaged 1500 and 350, respectively. The remaining laboratories analyzed fewer than 60 specimens per week (range from 13–60).

Inappropriate behaviors associated with an obvious risk for cutaneous contact with blood were identified at nearly 40 percent (15/39) of the areas surveyed. One example of these inappropriate work practices which occurred was that workers routinely handled contaminated fomites without wearing gloves. Despite the presence of warning labels (e.g., blood and body-fluid precautions) on some specimens, workers at 60 percent (9/15) of these same areas were not motivated to wear gloves. Workers who consistently wore gloves when working with blood (whether a warning label was present or absent) were observed at only 33 percent (13/39) of the stations. Surprisingly, two workers who routinely analyzed blood donned gloves only when they handled blood with a warning label.

Four workers committed flagrant violations of good work practices during the observations. These individuals either mouth-pipetted serum or live virus suspension or splashed milliliter amounts of body-fluid onto their skin. They were also observed to disregard basic hygienic practices (e.g., placing laboratory marking pens in the mouth) and misused equipment. Examples of other unsafe work practices observed in this study included: sonicating infectious materials without physical containment and hand braking a centrifuge rotor. Curiously, three employees acknowledged that they were aware of the risks associated with their actions.

Table 3 shows the frequencies and crude odds ratios of HBsAg contamination in 39 high-risk work stations. High work load and flawed technique were associated with the greatest risk of contamination, whereas there was a borderline association with inappropriate behavior (Table 3). Flawed techniques were of two kinds: those related to instrument design and those related to procedures which were intrinsically defective (i.e., workers may have been following the procedure as described, yet blood spills routinely occurred).

Approximately 85 instruments that workers used during the study were sampled for HBsAg. The design of these instruments was considered flawed if a mechanical feature generated a spatter of blood or necessitated frequent maintenance of components contaminated with body-fluids. HBsAg was detected on surfaces near several instruments where blood was spattered: Sequential Multiple Analyzer and Computer, Model SMAC II Systems: Technicon Instruments Corporation, Tarrytown, NY; Automated pH/Blood Gas System, Model 175: Corning Medical and Scientific, Medfield, MA: Pentawash II: Abbott Laboratories, Chicago, IL; and Coulter Counter, Model S-Plus System, Coulter Electronics, Inc., Hialeah, FL. Similarly, HBsAg was detected on instruments designed with samplers that required external washing. Compared with all other types of instruments surveyed in our laboratories, the continuous flow analyzers were the most vulnerable to malfunctions of

TABLE 2—Summary of HBsAg Environmental Sampling Data

		HBsAg Reactive			
		Initial Screen		Confirmed	
Laboratory No.	No. Tested[a] %[b]	No.	%[b]	No.	%[b]
1	87	0	0.0	0	0.0
2	245	44	18.0	21	8.6
3	139	11	7.9	2	1.4
4	46	1	2.2	0	0.0
5	90	0	0.0	0	0.0
6	19	0	0.0	0	0.0
7	14	0	0.0	0	0.0
8	45	10	22.2	8	17.8
9	47	1	2.1	0	0.0
10	68	2	2.9	0	0.0
Total	800	69	8.6	31	3.9

[a]No. = Number.
[b]Percent of total tested.

TABLE 3—Comparison of Factors with HBV Contamination in Laboratory Work Stations Assigned to the High-Risk Category

Factor	HBsAg Survey: Number of Stations with HBsAg/Total	Odds Ratio and 95% Confidence Interval
Work load (sera/wk)		
≤150	3/24	
>150	8/15	8.00 (1.55, 31.19)
Behavior		
Appropriate	4/24	
Inappropriate	7/15	4.38 (0.97, 16.61)
Containment Measures		
Appropriate Safeguards	2/22	
Flawed Techniques	9/17	11.25 (1.84, 46.06)
Complexity (manipulations/task)		
<30	10/29	
≥30	1/10	0.21 (0.04, 1.92)

intricate serum-containing components. All of these design features were associated with an increased occurrence of contamination in our laboratories.

Instruments with discrete reaction containers and samplers with fluid sensors that do not require external wash steps minimize contamination. Visible soiling was not observed and HBsAg surveys were negative after operating the following instruments: Automatic Clinical Analyzer III: Dupont Instruments, Wilmington, DE; Automated STAT/ Routine Analyzer-8: Beckman Instruments, Brea, CA: Immunochemistry Systems Analyzer II: Beckman Instruments, Brea, CA; and Cobas-BIO: Roche Analytic Instruments, Inc., Nutley, NJ.

Techniques which frequently resulted in blood spills, despite observations that workers adhered to written procedures, were considered defective because these methods did not reasonably ensure containment of biohazardous fluids. They were frequently associated with a need for repetitious separations and intermittent mixing of blood without adequate measures to contain spills. Both image and flow cytometric systems were identified as techniques with the most frequent occurrence of overt spills. These types of systems were associated with the presence of HBsAg in Laboratories 2, 3, and 8.

To approximate the risk of contamination when processing blood contained in vacuum-sealed tubes, samples were collected from a device designed for removing rubber stoppers from tubes. Operation of the device entails aligning the tube into an opening and, then, mechanically directing a metal arm to swing against the stopper forcing it to pop off simulating the manual technique. HBsAg was detected repeatedly on the rim of the device opening where spatter occurred. These data suggest that removing stoppers by either method is a subtle source of contamination. We endorse the technique of "wrap and snap" to minimize spatter of blood.

Tasks with few manipulations usually involved processing small volumes of blood using microscale techniques (both manual and automated). Automated instruments were used in 8 of 15 stations surveyed in Clinical Pathology compared with only 2 of 11 stations in Transfusion Medicine. Exclusive of the flow cytometry service, only manual techniques (e.g., immune profiles) were observed in the remaining research laboratories. Automated assays using less than 15 ml of blood averaged three manipulations per task: whereas, comparable manual assays averaged 10 manipulations per task. Tasks using greater than 1 L of blood required the most handling (average 30 manipulations/task: range from 6–92). These latter procedures were typically performed in research laboratories.

To examine the association between different tasks and contamination, we arbitrarily grouped each work station into those involving less than 30 manipulations and another group with 30 and more activities (complex tasks). In contrast to the factors mentioned earlier, complexity appeared to be inversely associated with contamination (OR = 0.21, 95% CI = 0.04, 1.92) (Table 3). A review of the data revealed that complexity was inversely correlated with work load (no complex tasks were performed in stations with high work load).

To further examine the predictors of blood-borne contamination, logistic regression analysis was performed to determine the effects of the factors shown on Table 3 while controlling for the effects of each other. Because of the negative correlation between complexity and high work load, we chose to remove complexity from further analysis. In the analysis with the three remaining factors, the point estimates for the adjusted odds ratio for flawed technique decreased to 9.78 (95 percent CI = 1.46, 65.49) and was the factor most strongly associated with HBsAg. Only a modest decrease in the adjusted odds ratios was observed for high work load (OR = 5.06, 95% CI = 0.80, 31.96) and inappropriate behavior (OR = 2.75, 95% CI = 0.44, 17.4) compared with the crude odds ratios. In a similar analysis with only two variables, both high work load (OR = 6.82, 95 percent CI = 1.73, 39.66) and flawed technique (OR = 9.78, 95 percent CI = 1.52, 63.01) were associated with an increased risk of blood-borne contamination. (Data available on request to authors.)

Discussion

Data from our study are consistent with the concept that workers who process blood from numerous donors (high work loads typically associated with diagnostic testing) increase their risk of occupational exposures to blood-borne pathogens. However, a high prevalence of HBV infection among blood donors can also contribute to risk. For example, the liver disease research unit had the highest HBsAg positivity rate (17.8 percent) but only averaged 27 specimens/ week. One-third of the specimens submitted to this laboratory were from individuals known to be chronic carriers of HBV, compared with the other laboratories surveyed where fewer than one-tenth of the specimens were obtained from infected patients. It is important to note, however, that an analogous risk can be identified in any laboratory where serial specimens from infected patients are analyzed (e.g., therapeutic drug monitoring, diagnostic microbiology, and similar types of research involving HBV or HIV). In support of this observation is the recommendation that the function or nature of the laboratory should also be considered in the hazard assessment to protect these workers.[8]

Inappropriate behaviors (a second factor associated with HBsAg) were noted when workers disregarded standard practices or misused equipment. Somewhat alarming were observations that individuals at almost 50 percent of the high-risk areas experienced a seemingly innocuous, but preventable cutaneous contact with blood as a result of poor work practices. Complacency regarding the wearing of gloves while handling contaminated fomites was cited as the most frequent cause of these exposures.

During 75 hours of observation, only four overt accidents resulting in contact between the employees' protective garb and blood were observed. No accidental parenteral inoculations occurred and none of the four accidents was associated with inappropriate behaviors. These data can be contrasted with our observations cited above that poor work practices were associated with the employee experiencing cutaneous contact with blood. Based on these data, we concluded that the majority of exposures to blood that occur in the laboratory are occult in nature, as well as, preventable. Further, the risk of processing blood containing a pathogen is likely to be underestimated.[18] Despite the presence of warning labels on specimens submitted to NIH laboratories, during our observations, the majority of workers who engaged in poor work practices were not motivated by these labels to modify their inappropriate behaviors. Equally important was our observation that none of the individuals with good work practices experienced an adverse exposure to blood. In combination, these data reinforce the concept that workers who handle human blood strictly adhere to precau-

tions as an effective means to reduce their risk of laboratory-associated infection. These practices were observed prior to the implementation of universal precautions in our hospital.[12]

Our results indicate that automation contributed to contamination only when the design of certain instrument components was flawed with respect to containment.[19] Just as good engineering design can be negated by poor work practices, flawed design (the factor associated with the greatest risk of contamination) can also overcome good work practices.[9] Our study demonstrates the need to modify instrument components which represent an increased risk for contamination and, perhaps, infection among laboratory workers.

Manual techniques such as cytometric systems that involved sequential manipulations of specimens represented an increased risk for contamination in our laboratories. The frequent presence of HBsAg in these areas indicates that blood-borne contamination is predictable for many of these procedures. We concur that workers should modify these techniques in order to enhance their safety.[9]

In our study, the complexity of laboratory tasks (in terms of cumulative manipulations per task) was weakly associated with the risk for HBsAg. Also, we observed a negative correlation between complexity and high work load which probably reflected differences in the processing of specimens for research protocols compared to diagnostic testing. We submit, therefore, that the complexity of tasks performed represents minimal risk to the worker and recommend that predictors associated with greater risk of blood-borne contamination be given priority in the assessment of laboratory safety. Use of these data and previously published data[2,8,12] in designing interventions to eliminate adverse exposures to blood may prevent implementing strategies that unnecessarily disrupt the laboratory while not enhancing worker safety.

APPENDIX A

Method Used for HBsAg Confirmation Assay

Modifications to the manufacturer's instructions (Ausria II Confirmatory Neutralizing Kit: Abbott Laboratories, Chicago, IL) were as follows. The HBsAg positive control (Ausria II: Abbott Laboratories, Chicago, IL) was processed in the same manner as the samples. After the addition of a polystyrene bead coated with guinea pig anti-HBsAg, 150 μl of eluate or HBsAg positive control were dispensed to each of four wells. The negative control (FBS/PBS) was assayed in replicates of six using 150 μl per well. After the initial wash step, 150 μl of FBS/PBS (diluted 1:5 with 0.9% NaCl) were added to the negative control wells. Each tray was incubated (25°C for 16–24 h). Each well was then washed four times using 5 ml distilled water per rinse. A 150 μl volume of confirmatory anti-HBsAg (diluted 1:5 with 0.9% NaCl) were added to two of the four wells and 150 μl of FBS/PBS (diluted 1:5 with 0.9% NaCl) were added to the remaining two wells of each set of assays and to each of the negative control wells. Each tray was incubated (45°C for 1 h). Without aspirating the contents of the well, 200 μl of I-125 anti-HBsAg were then added to all of the wells and allowed to react (45°C for 1 h). After repeating the wash step, each bead was counted.

The average cpm was calculated for each replicate assay that reacted with either anti-HBsAg or FBS/PBS. The percent reduction in the cpm for the positive control and each sample was calculated using the following formula:

$$\% \text{ Reduction} = \frac{\{\text{cpm(sample + FBS/PBS)} - \text{cpm(sample + anti-HBsAg)}\} \times 100}{\{\text{cpm(sample + FBS/PBS)} - \text{cpm(negative control)}\}}$$

Validity of a confirmation run was based on two arbitrary criteria: 1) neutralization with anti-HBsAg had to result in a greater than 50 percent reduction in the cpm for the positive control when compared to the cpm of the control reacted with I-125 anti-HBsAg (the parameter was >97 percent for each run); and 2) no more than two replicates of the negative control could be considered aberrant. This latter event did not occur. However a single value was greater than 1.5 times the mean of the negative control for three of the five runs. In each case, the aberrant value was excluded and the mean recalculated.

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