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Detection of hemoglobin peroxidase activity in the dental suite and its removal from inanimate environmental surfaces

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DETECTION OF HEMOGLOBIN PEROXIDASE ACTIVITY IN THE DENTAL SUITE AND ITS REMOVAL FROM INANIMATE ENVIRONMENTAL SURFACES

by

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I INTRODUCTION

Dental health care workers (DHCWs), the dental operatory and surrounding environment are exposed to blood, saliva and particulate debris.[1] Because of this exposure to potentially infectious materials, DHCWs are susceptible to blood and saliva-borne infections by aerosols and direct contact with infectious agents.[2-5] DHCWs can acquire diseases such as hepatitis B,[6-13] herpes simplex,[14-29] tuberculosis,[30] and pneumonia.[31] Infected individuals can experience debilitating and chronic disease outcomes which can limit work practices.

Environmental surfaces contaminated with patients' oral fluids may be a source of infectious organisms. Several studies have demonstrated that infectious organisms can remain viable on inanimate surfaces. Herpes simplex virus (HSV) can survive for over four hours on patient charts.[32] Hepatitis B (HBV) and the human immunodeficiency virus (HIV) can remain viable in the desiccated state for at least seven days.[33-34] Table 1 demonstrates the stability of herpes simplex virus on patient charts over a period of several hours.[32]

Table 1: Testing of saliva on dental charts for bacterial viability

Time*	Growth†
30 minutes	++++
60 minutes	++++
2 hours	++++
24 hours	+++
48 hours	++
3 days	+
4 days	+
5 days	+
6 days	0
7 days	0

*Time after chart inoculation
†Growth rate by colony count: ++++ = greater than 15 colonies/plate, +++ = 10 to 15 colonies/plate, ++ = 5 to 10 colonies/plate, and + = 1 to 5 colonies/plate.

Thomas LE et al. Survival of herpes simplex virus and other selected microorganisms on patient charts: potential source of infection. J Am Dent Assoc. 1985. 111(3); 461-64.[32]

Because many organisms may remain viable on environmental surfaces in the dental suite the latter may serve as a source of infection. It is important to determine the potential extent of microbial contamination in the dental environment. An obvious microbial source is the patient's oral fluids. Trace amounts of blood, saliva, and gingival exudates are difficult to see. If blood and other oral fluids are not seen, then the areas they cover may not be routinely decontaminated and if those surfaces are not properly decontaminated, may represent a hazard to the practitioner or patient.

To date, there has been limited knowledge about the dispersal of the patient's oral fluids to surfaces in the dental environment and in our ability to assess the removal of these fluids from dental surfaces by commonly-used dental

disinfectants.

There are several methods by which environmental surfaces can be assessed for oral fluids. These methods include simulation studies which substitute a colored dye for oral fluids and determine the dispersal of the dye by performing treatment procedures on mannequins, determining microbial dispersal of oral microorganisms, determining the dispersal of salivary proteins, and determining the dispersal of blood, among other methods. Blood can be detected on environmental surfaces by microscopic evaluation or by detection of hematologic components such as hemoglobin. For this study, we use the detection of hemoglobin, specifically hemoglobin peroxidase activity (HPA), as an environmental assessment for blood.

The purposes of this study were to determine (1) the sensitivity and reliability of a reagent strip which detects hemoglobin peroxidase activity, and to define a sampling technique; (2) the cross reactivity of common dental disinfectants with the reagent strips; and (3) the removal of hemoglobin from dental surfaces with a dental disinfectant.

BACKGROUND

Infectious diseases: hepatitis B, herpes simplex, and HIV infection

The three most significant bloodborne pathogens in the

dental setting are hepatitis B virus, herpes simplex virus, and human immunodeficiency virus. Diseases caused by the pathogens, risk of transmission and risk of occupational infection in the health care environment are discussed below.

Hepatitis B

Hepatitis B infection can range from asymptomatic or mild to serious and life-threatening. Hepatitis B virus (HBV) may be found in several body fluids including urine, tears, semen, vaginal secretions and breast milk.[35] However, only blood, semen and saliva have been shown to transmit infectious virus.[36-37] The most likely source of virus in the oral cavity is crevicular fluid.[36] HBV is transmitted by percutaneous or mucous membrane exposure to blood and blood products. Common, documented modes of transmission include exposure to blood and blood products by needlestick injury, and conjunctival or mucous membrane exposure. Approximately six to ten percent of all hepatitis B cases progress to a carrier state, in which hepatitis B surface antigen (HBsAg) circulates indefinitely in the blood.[38] Consequences of chronic infection include increased risk for hepatocellular carcinoma and cirrhosis of the liver as well as immune complex disorders including serum sickness syndrome, mixed cryoglobulinemia, glomerulonephritis, and vasculitis.[5] Individuals are

considered to be infectious when experiencing an acute infection or when they are in an established carrier state.[39] The presence of specific antigenic markers such as hepatitis B e antigen (HBeAg) is associated with infectivity. Individuals carrying HbeAg tend to be more infectious than those not carrying the marker.[11]

Hepatitis B infection among health care workers (HCWs) is a serious occupational hazard. HBV infection can occur after percutaneous injection of dilutions as low as 1:1,000,000.[40] Oral transmission has also been documented.[41] The overall risk of hepatitis B among health care workers is estimated to be approximately four times that of the general adult population.[10,42-43] Table 2 demonstrates that health care workers, physicians and dentists are five to ten times more likely to experience hepatitis B infection compared to the general population.[10]

Table 2: Incidence of HBV in physicians and dentists.

Profession (specialty/affiliation)	Number of Cases or Infections	Incidence (events/1000 person-years)	Relative Incidence	Reference
Physician (dialysis units)	8	14	11.7	Snydman et al.[44] 1975
Physician (US Army)	9	19.7	16.4	Segal et al.[45] 1979
Physician (hospital staff)	7	23	19.2	Levy et al.[32] 1977
Physician (hospital staff)	4	4.1	3.4	Pantelick et al.[46] 1981
Physician (hospital staff)	19*	2.5	2.1	Osterholm & Andrews Jr.[47] 1979
Physician (hospital staff)	18	11.0	9.1	Polesky & Hanson.[48] 1980
Dentist	5†	5.6	4.7	Mosley & White.[49] 1975
Dentist	38	25.3	21.1	Mosley & Edwards.[26] 1981
Dentist (US Army)	1	6.2	5.2	Segal et al.[45] 1979
Dentist (4th year students)	3	27.2	22.7	Goebel & Gitnick.[29] 1979

*Several of the cases included here may also have been reported in the study of Levy et al.[32] 1977.
†Based on self-reported cases that were not serologically confirmed as type B.

From West DJ. The risk of hepatitis B infection among health professionals in the United States: a review, The Am J Med Sci. 1984: 287(2); 26-33.[10]

In the dental health care worker population, the risk of an established carrier state ranges from three to ten times that of the general population.[44]

Hepatitis B infection among DHCWs is probably influenced by the degree of blood exposure.[10] Table 3 shows that HBsAg infection occurs more frequently among oral surgeons, a speciality which most likely experiences the most blood exposure, in comparison to the total dental population.[13,44]

Table 3: Combined data on the prevalence of markers for HBV in health care personnel.

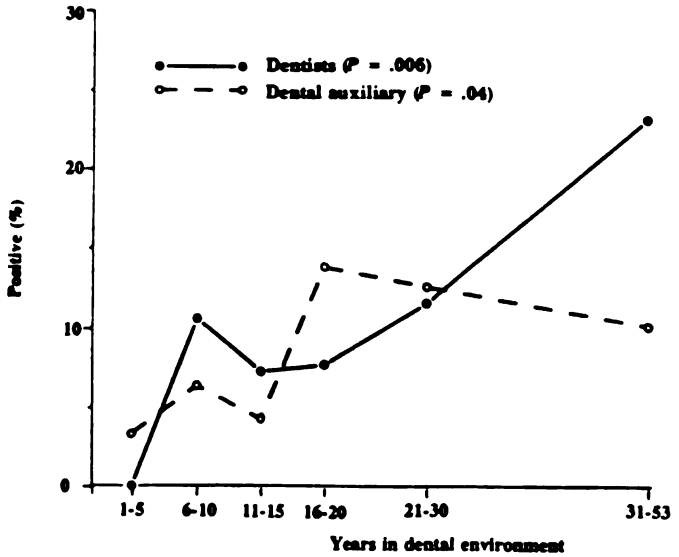
	Prevalence of HBV markers (%)	Annual attack rates (%)
General physicians	12 to 19	2
Medical house officers	8 to 10	. . .
Family practice	15 to 16	. . .
Internal medicine	14 to 18	
Surgeons	20 to 28	5
Surgical residents	10 to 17	4 to 10
Pathologists	20 to 27	
Anesthesiologists	17	
Obstetricians/gynecologists	14 to 16	
Pediatricians	13 to 21	
Oral surgeons	30	5
Dentists	14 to 15	2
Psychiatrists	3	
Nonpatient care	4 to 8	

References: Smith and others[6]; Mosley and others[16]; Dienstag and Ryan[17]; Maynard[18]; Denes and others[19]; and Tayler and others.[20]

Cottone JA. Hepatitis B virus infection in the dental profession. Proceedings of the National Symposium on Hepatitis B and the Dental Profession. J Am Dent Assoc. 1985: 110; 617-21.[44]

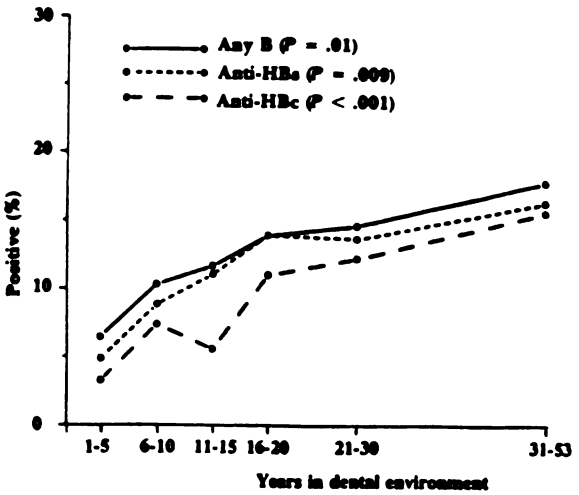
The prevalence of HBsAg antibody is directly related to the age of the practitioner.[45] Figure 1 and 2 indicates that, at least in one study, the likelihood of having positive markers for HBV infection increases with the number of years of practice.[43]

Figure 1: Relationship of years of practice in a dental environment with positive anti-HBc for dentists and practicing staff.



Schiff ER et al. Veterans administration cooperative study on hepatitis and dentistry. J Am Dent Assoc. 1986: 113(3); 390-96.[43]

Figure 2: Relationship of years of practice in the dental environment with positive markers for HBV infection for dentists and practicing auxiliary.



Schiff ER et al. Veterans administration cooperative study on hepatitis and dentistry. J Am Dent Assoc. 1986: 113(3); 390-96.[43]

Herpes simplex

Herpes simplex (HS), caused by herpes virus hominis, occurs in two antigenically distinguishable forms. Type 1 is associated with infections of the oral mucosa, skin and conjunctiva while type 2 is associated with genital infections. Clinical manifestations include acute gingivostomatitis, keratoconjunctivitis, and herpes simplex of the skin.[26] Following the primary infection, the virus may persist in the trigeminal or sacroiliac dorsal root ganglia and in the Schwann cells of sensory nerves which supply the infected area. Recurrent attacks can occur and secondary infection is common.[24] Herpes simplex virus (HSV) can be found in the herpetic lesions, saliva and tears.[24] HS is common among the general population. At least ninety percent of the adult population have neutralizing antibodies to HSV. HS is a significant occupational hazard and can be a debilitating infection to health care workers after inoculation of the hand, eyes, or percutaneous inoculation. Infections of the eye can occur by splashes of infectious virus into unprotected mucous membranes of the eye. Infection of the hand is most likely associated with breaks in the skin. Table 4 demonstrates that health care professionals such as physicians and nurses are susceptible to acquiring an infection by percutaneous routes such as needlesticks or exposure through breaks in the skin.[46]

Table 4: Incidence and history of herpes simplex in health care workers.

Patient	Hist. of Previous Herpes Simplex Infect.	Hist. of Trauma at Site	Hist. of Giving Mouth and Oro-Pharynx Care	Onset of Skin Lesions After Injury	Regional Lymphadenopathy	Pain
1 Surgical resident	Negative	Surgical laceration of finger	Yes	7 days	Present	Present
2 Student nurse (Fig 1)	Negative	Nail-biting	Yes	Not determinable	Present with lymphangitis	Present
3 * Student nurse (Fig 2)	Negative	Cuticle-biting	Yes	Not determinable	Present	Severe
4 Surgical resident	Negative	Needle puncture of skin	Yes	3	Present with lymphangitis	Severe
5 Student nurse	Negative	Needle puncture of skin	Yes	3	Present	Present
6 Student nurse (Fig 4)	Negative	Laceration of skin by ampule	Yes	4	Present	Present

* Presented previously at Philadelphia Dermatological Society, Feb. 14, 1957.
N.D. - Not Done

Hambrick GW et al. Primary herpes simplex infection of fingers of medical personnel. Arch Dermatol. 1962: 85(5); 583-89.[46]

DHCWs have at least a two fold increased risk of acquiring HS in comparison with the general population.[18,47] The most likely mode of transmission of HSV to dental health care workers is by direct contact of non-intact skin with an infectious lesion or fluids. The most common documented site of infection in DHCWs is an infection of the finger, herpetic whitlow.[16,18,19,24-25,27-28]

Human immunodeficiency virus

The acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), is an infectious disease transmitted by exposure to blood or certain other body fluids. HIV has been isolated from blood, semen,

saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk and cervical secretions.[48] Transmission is known to occur after exposure to blood, blood products, semen, cervical secretions, and breast milk.[48] Infectious HIV may be present in saliva,[49,50] however this is a rare occurrence and to date there has not been any documented cases of transmission by saliva.[50] Once infected, an individual with HIV usually experiences one or more opportunistic infections leading to fatal outcomes. Currently, a vaccine for the prevention of HIV does not exist. Neutralizing antibodies which usually confer immunity for viral infection may be helpful in blocking initial infection by free virus. However once the infection is established, neutralizing antibodies do not appear to change the course of the disease.[48]

HIV infection is an occupational hazard to health care professionals because it can be transmitted by exposure to blood and body fluids.[51] HIV has been transmitted to health care professionals by needlestick, mucous membrane contact and, in one case, breaks in skin.[51] Defining the risk of health care workers is complicated. The risk of a HCW acquiring HIV may be influenced by a variety of factors such as size of inoculum, extent of exposure, and susceptibility of the individual. However, it is generally agreed that all health care workers who are exposed to blood and or body fluids are susceptible to the disease. Prospective studies

have estimated the risk of occupational acquisition for DHCWs to be less than 1%.[52-54]

One dentist has been reported as possibly having been occupationally infected with HIV.[54] It was suggested that the infection was transmitted by needlesticks or breaks in the skin. However, documentation is incomplete and it could not be specifically determined if the infection was occupationally derived. Currently, it is believed that the most important mode of occupational transmission may be by accidental needlesticks or sharp instrument wounds.

Transmission of infectious diseases to patients

Surveillance studies have demonstrated transmission of infectious diseases such as hepatitis B or herpes simplex,[55] to patients by infected DHCWs.[45,56] In one outbreak of hepatitis B, traced to a dentist, two patients died of fulminant hepatitis B.[57] Since none of the dentists associated with this outbreak used gloves during dental or surgical procedures, it was likely that transmission occurred through breaks in the skin of the dentists' hands.[8] Invasive dental procedures were associated with a greater likelihood of the patient acquiring HBV infection.[47] Table 5 and 6 indicate the association between specific dental procedures and HBV infection.

Table 5: Association between dental procedures and HBV infection in patients treated from 3/1/84-9/8/84, Indiana.

Procedure	% of Cases Exposed (N=23)	% of Controls Exposed (N=588)	Odds Ratio	95% Confidence Interval
Filling	52 2	48 4	1 2	0.47-2 87
Crown	30 4	8 1	4.9	1.76-13.45
Periodontal treatment	30 4	49 7	0 4	0.16-1.16
Extraction	26 1	5 7	5.9	1.94-17.01
Surgery	21 7	0 9	31 6	7.48-132.8
Denture fitting	21 7	5 8	4.5	1.38-13.77
Observation	26 1	10 6	3.0	1.01-8.34
Prophylaxis	4 3	17 7	0.2	*-1.49

*Lower limit could not be calculated because of small numbers

Shaw FE et al. Lethal outbreak of hepatitis B in a dental practice. J Am Med Assoc. 1986: 255(23); 3260-64.[57]

Table 6: Multivariate analysis of dental procedures and HBV infection in patients treated from 3/1/84-9/18/84, Indiana.

Procedure	Crude Odds Ratio	Adjusted Odds Ratio*	95% Confidence Interval
Surgery / extraction	7 4	3 62	1.31-10 00
Crown	4.9	4 28	1.84-11.15
Denture	4 5	2 06	0 62-6 90
Observation	3 0	1 68	0 60-4 69

*Logistic regression adjustment for other variables

Shaw FE et al. Lethal outbreak of hepatitis B in a dental practice. J Am Med Assoc. 1986: 255(23); 3260-64.[57]

Environmentally-mediated infections

There is concern that infectious diseases can be acquired from contact with surfaces, equipment or instruments bearing infectious organisms. It has been demonstrated that some fomites have prolonged viability, which may be influenced by the presence of organic matter

such as protein. Herpes simplex virus is viable longer when blood is present.[32] A protein coating also influences the effectiveness of disinfectants to reduce bacterial counts.[58]

Infections such as hepatitis B have been associated with inanimate surfaces bearing infectious organisms and minor skin abrasions or contact with mucous membranes.[35] Transmission of HBV has also been associated with computer card handling, toothbrush racks, coffee cups, baby bottles and equipment surfaces in hemodialysis centers.[35,37,41,59] Contaminated environmental surfaces have been implicated as a likely source of post-surgical infection from arthroscopic surgery.[60] The etiologic agent isolated from the patients' infection was also present on the instrument table and glutaraldehyde container in which the arthroscope had been placed.

Environmental surface assessments for infectious agents

There are several methods of assessing environmental surfaces for infectious agents. These methods include microscopic evaluation, which detects markers of the agent such as the hepatitis B surface antigen (HBsAg), and evaluating the viability of infectious agents on inanimate surfaces.

Microscopic examination can reveal the presence of red blood cells or microorganisms. Molinari et al used tracer bacterial organisms in blood to detect the effectiveness of

dental disinfectants in removing blood and deactivation of the tracer bacteria which was placed onto various inanimate surfaces.[61]

Detecting antigenic markers for infectious organisms such as determining the presence and viability of the marker HBsAg for the hepatitis B virus is another type of environmental assessment.[37],[62] However, it is difficult to associate the transmission of some pathogens with the presence of infectious organisms on inanimate environmental surfaces.

The dispersal of oral fluids in the dental setting

There are several methods for assessing the dispersal of oral fluids which include determining the dispersal of aerosol particles,[63,64] blood, microbial dispersal,[65] and simulating the dispersal of oral fluids by using colored or fluorescent dye[66] in the dental setting.

Molinari et al simulated the spread of oral fluids during a normal treatment procedure.[66] Fluid dispersal simulated by a colored dye demonstrated that the dye transferred to the practitioner's mask and glasses and to surfaces in the operatory environment. Equipment and objects such as bulk dispensers of dental material, the air/water syringe, handpieces and light handle demonstrated the transfer of the dye. Demonstrating the transfer of the patient's oral fluids to surfaces in the dental operatory by

simulation studies shows that contact with patient fluids is unavoidable.[66] Simulation studies also demonstrated that the total biological load or bioburden on environmental surfaces is difficult to see.

Objects placed directly into the patient's mouth such as radiographic equipment, handpieces, air/water syringes, and gypsum casts could be a vehicle for infection between patient and personnel.[65,67] A point source epidemic of Mycoplasma pneumoniae in prosthetic laboratory employees was traced to a prosthetic appliance.[31] Handpieces and air/water syringes have higher levels of microbial colony forming units (CFUs) compared to CFUs cultured from tap water in the same office.[68-69] Table 7 shows the amount of colony forming units of bacteria isolated from dental units.

Table 7: Bacterial contamination of water supplied to and isolated from dental units.

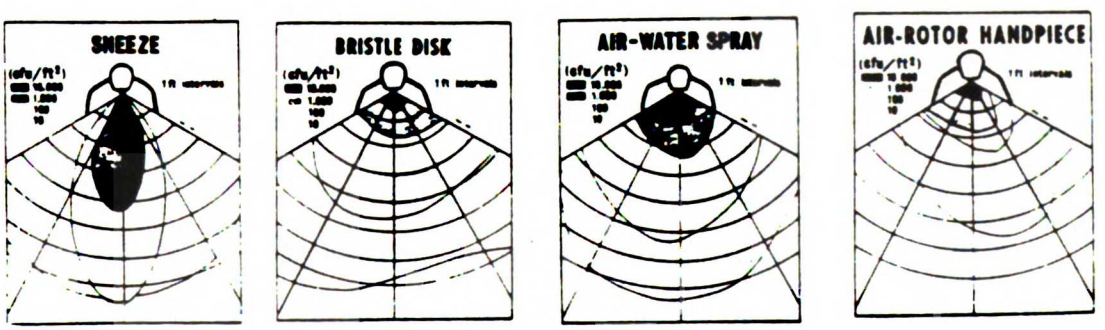
Clinics investigated	Water sampling points	No. of tests*	Mean no. of bacteria (cfu ml)
Dental clinic K	Tap water flowing to sink	1	40
	Cup-water filler-nozzle	10	80
	Three-way syringe	10	10^3
	Air-turbine handpieces	10	10^4
Dental clinic K.I.	Tap water flowing to sink	1	0
	Three-way syringe	3	10^3
	Air-turbine handpieces	3	10^4
Dental clinic 0	Tap water flowing to sink	1	0·04
	Cup-water filler-nozzle	14	10^2–10^3
	Three-way syringe	14	10^4
	Air-turbine handpieces	14	10^4
Dental clinic S	Tap water flowing to sink	1	0
	Three-way syringe	2	10^3–10^4
	Air-turbine handpieces	2	10^4
Dental clinic Y	Tap water flowing to sink	1	0·04
	Three-way syringe	3	10^3
	Air-turbine handpieces	3	10^4
Dental University Hospital T	Tap water flowing to sink	1	0
	Cup-water filler-nozzle	10	0
	Three-way syringe	10	10^3–10^4
	Air-turbine handpieces	10	10^4

* The number of tests (except tap water flowing to sink) represents the number of dental units tested

Furuhashi M et al. Prevention of bacterial contamination of water in dental units. J Hosp Infect. 1985: 6; 81-88.[68]

Rotary instruments with water spray generate aerosolized particles of infectious organisms.[67,70-71] These particles in turn are dispersed in the clinical environment. Air turbine handpieces produce at least 7,000 colony forming units per minute more when compared to common naso-oral activities.[72] Bacterial aerosol particles in the range of 0.5 to 10 μm in diameter are of particular interest because these particles can be inhaled and lodged in the terminal bronchioli and alveoli.[65,67,72] Airborne particles of this size also remain suspended in the environment as demonstrated by streptococcal aerosol particles remaining airborne and viable for over 24 hours.[65] Miller et al demonstrated that bacterial organisms can travel in an air conditioning system and can be recovered from the air and nasopharynx of occupants (Figure 3).[73]

Figure 3: Concentration and dispersal of bacterial particles produced from a sneeze and during the use of various equipment in the dental operatory.



Miller RL. Studies of the aerobiology of dentistry. Airborne transmission and airborne infection. Editors Hers JFPh and Winkler KC. John Wiley and Sons. New York-Toronto. 1973: 494-503.[73]

While it is important to consider the influence of aerosols as an occupational hazard, the study of aerosols generated during dental treatment is limited to the measurement of particulate size and microbial dispersal. Risk assessments of the environmental hazards in the dental suite should consider aerosols and splatter generated during treatment. However, the role of aerosols in relationship to occupational risk is limited. Aerosols can be significantly reduced if the DHCW practices appropriate precautions. Precautions such as using a rubber dam and high volume evacuation can reduce the amount of aerosols released into the environment over ninety-nine percent.[64,68,73]

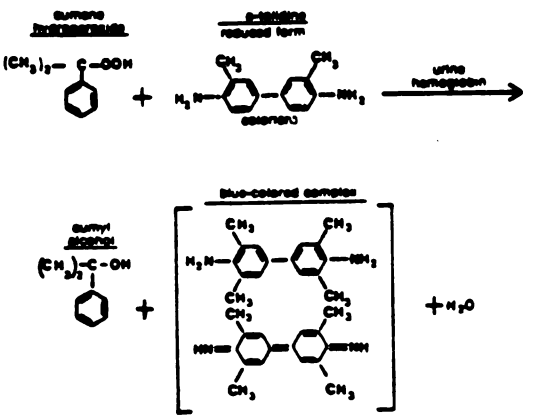
Environmental assessments for blood

On June 24, 1988 the Centers for Disease Control (CDC) stated that "blood is the single most important source of HIV, HBV and other blood borne pathogens in the occupational setting."[74] Detecting the presence of blood on inanimate surfaces in health care settings may be an appropriate risk assessment. Previous studies have demonstrated the transfer of blood from the patient onto the orthopedic surgeon's protective glasses during surgical procedures.[75] The use of rotary tools which produce a forced spray were most likely to produce visible blood on eyewear. It is likely that rotary dental equipment will create the same dispersal pattern. Visualization of blood is probably not the most

accurate assessment of occupational exposure because the presence of occult blood (blood which is not visible) cannot be seen.

A reagent test strip can be used to detect the peroxidase-like activity of hemoglobin.[62,76-79] The strips can detect hemoglobin in whole red blood cells and also hemolyzed blood.[78-79] A reagent strip consists of tetramethylbenzidine, cumene hydroperoxide, and methyoxyquinoline and buffers mixed and impregnated onto a small absorbent square. This square is subsequently attached to a plastic strip, hence the reference to **test strips** or **stick test**. When hemoglobin is present, the oxidation of tetramethylbenzidine by the peroxide results in a blue color change. The reaction is catalyzed by hemoglobin (Figure 4).[77]

Figure 4: Chemical reaction of reagent strips which test for occult blood.



Jackson JA. Technical aspects of urine dipstick reagent areas. Clin Prod Rev. December 1985: 4(12); 10-19.[77]

Strip tests or similar chemical reactions have been used to detect the presence of hemoglobin on surfaces in an autopsy room, on the gloved hands and instruments of central sterile supply personnel, and on surfaces in a dialysis center.

A study which assessed the frequency and origin of blood on medical records demonstrated blood present on 1.5% of all laboratory reports and the blood was present before the reports reached the laboratories.[80] Kennedy et al detected blood on 15% of the gloved hands of central sterile supply personnel in a hospital setting and that blood was present on 59% of cleaned instruments.[81] Table 8 shows the total and percent prevalence of blood on the hands and cleaned instruments in the central supply area.

Table 8

Hands			Instruments		
No. Tested	No. Positive	%	No. Tested	No. Positive	%
104	16	15	34	20	59

Kennedy PB et al. The detection of blood on gloved hands of central supply personnel and cleaned instruments used for procedures on patient unite. Infect Control Hosp Epidemiol. 1988: 9(3); 117-18.[81]

Although gloves may protect the practitioners from exposure to infectious agents, practitioners may be unaware that they have been exposed to infectious agents on surfaces not normally considered contaminated. If the practitioner

assumes that a surface is free of infectious agents because it is not involved in direct treatment procedures or is considered free of infectious fluids, the practitioner will not take the necessary precautions.

Examination of an autopsy suite detected blood on surfaces which did not have direct contact with cadavers and body tissues such as refrigerator door handles, desk tops, cabinet knobs, phones, and door knobs.[82] Because these surfaces did not have direct contact with cadavers and body tissues, they were excluded from routine cleanup procedures. It was determined that the presence of blood was associated with a deterioration of infection control practices.[82] Table 9 lists the specific surfaces on which blood was found in the autopsy suite.

Table 9:

Cold water faucet handles	Desk top
Hot water faucet handles	Cabinet knobs
Oscillating bone saw handle	Phone receiver
Disinfectant bottle	Drawer handles
Refrigerator door handle	Door knob
Formalin dispensing valve	

Beaumont LR. The detection of blood on nonporous environmental surfaces: An approach for assessing factors contributing to the risk of occupational exposure to blood in the autopsy suite. Infect Control. 1987: 8(10); 424-26.[82]

Hepatitis B, herpes simplex and HIV are among the most serious of bloodborne infections which can be or have the potential to be occupationally acquired. In the past,

infection transmission has occurred by exposure of susceptible individuals to infectious blood and certain body fluids. In the dental environment, dental health care workers and patients are exposed to potentially infectious organisms by the dispersal of the oral fluids onto surfaces, equipment and the practitioner themselves. Although most pathogenic organisms are fragile when outside of the host,[58,83-85] routine decontamination is recommended for items in contact with oral fluids. Heat sterilization is the method of choice for sterilization of those items which can withstand heat and moisture. Those items which cannot withstand heat or moisture are disinfected with a disinfecting solution.

Previous studies have examined the dispersal of oral fluids to surfaces in the dental environment, however, there is a lack of a routine environmental assessment to determine the dispersal of oral fluids and the extent of this dispersal. Although it will be difficult to develop environmental assessments because all dental offices are not structurally the same, determining the dispersal of oral fluids may help individual practitioners to trace the dispersal of oral fluids and to monitor the infection control practices of the office staff.

II MATERIALS AND METHODS

Reagent strips were utilized to develop an assessment for detecting hemoglobin in the dental suite. The reagent strips were tested for hemoglobin sensitivity and a method for inanimate surface sampling was developed. Various surfaces in dental operatories were sampled for the presence of hemoglobin. The reagent strips were tested for cross reactivity with common dental disinfectants after results from the data analysis of the surface sampling (conducted in dental suites) revealed that a high percentage of reagent strips were positive for the sampled surfaces. Whole human blood was placed on metal, cloth and counter top surfaces and treated with a common dental disinfectant. The ability of the disinfectant to remove blood was assessed using the reagent strips.

Phase 1: Testing the sensitivity of hemoglobin reagent strips and developing a sampling technique

Serial dilutions of whole human blood were performed to determine the sensitivity of a reagent strip which reacts with the peroxidase-like activity of hemoglobin. Whole human blood was placed into four microliter pipettes (one and five microliter pipettes). The contents of two pipettes, one and five microliters, were emptied into separate fifty milliliter glass vials which contained 1 ml of sterile saline. The remaining microliter pipettes were

dispensed onto separate glass slides. Each slide was swabbed with a sterile cotton swab moistened with sterile physiologic saline. The swab was placed on the glass slide, pulled across the slide lengthwise and rotated clockwise. The swabs were placed into fifty milliliter glass vials containing 1 ml of sterile saline. After approximately five minutes the swabs were removed. All four vials were agitated for thirty seconds. A regent strip (Hemastix, Ames - Mishikawa, Indiana) which tests for occult blood in urine was placed into each of the glass vials. The reagent strip was immediately withdrawn from the solution. Immediate withdrawal assures that the reagents on the strip are not dissolved from the strip. The color of the strip was read after forty seconds. The results were compared to six color blocks provided by the manufacturer of the reagent strips. Each block was assigned a number from zero to five. Table 10 shows the values which were assigned to each color block.

Table 10: Values recorded for color changes for reagent strip.

0	negative
1	non-hemolyzed
2	hemolyzed trace
3	small +
4	moderate ++
5	large +++

0.1 ml of the solution in each of the four test vials was placed into four separate test vials which contained 1.0 ml of sterile saline. The vials were agitated for thirty seconds and measured with the reagent strips as described above. The results were recorded. 0.1 ml of these vials were again transferred, measured and repeated one more time. The concentration of the final dilution was 1 x 10-6. A positive control was obtained by sampling undiluted whole human blood with the reagent strip. A negative control was obtained by sampling sterile saline with a reagent strip.

A technique using the reagent strips to measure hemoglobin peroxidase activity on inanimate surfaces was developed.

Sampling technique:

1. The reagent strip was moistened with sterile saline in a ten milliliter vial.
2. The strip was immersed in the saline and immediately withdrawn.
3. The strip was placed directly upon the surface of interest and lightly pressed with an index finger. The strip was immediately lifted off of the surface.
4. A negative control was obtained by sampling the sterile saline with a reagent strip. The positive control was an instrument tip with visible blood from dental treatment.

Phase 2: Surface testing

To determine if HPA was present on various inanimate surfaces in dental operatories, reagent strips were used to measure HPA from inanimate surfaces in dental operatories. Four dental clinics (University of California, San Francisco) were chosen for surface testing. The clinics were the predoctoral, oral surgery, periodontal surgery, and the faculty group practice. Ten randomly selected operatories were chosen in each clinic. Ten operatories were observed immediately prior to patient treatment (time 1) and ten operatories were observed immediately following patient treatment (time 2). Ten surfaces in each operatory were chosen for testing. The surfaces were:

1. the high volume evacuation system (HVE)
2. a handpiece (H/L)
3. a pen or pencil (pen)
4. the clinic telephone (phn)
5. protective eyewear (gls)
6. a sterile instrument (ins)
7. the connector to the high speed handpiece (con)
8. the master switch (sch)
9. the air/water syringe (A/W)
10. the faucet handle (fct).

If some surfaces were not present in all operatories, the surface was matched to a comparable surface. Scores for

all surfaces were recorded and compiled.

Appropriate human research consent was obtained from the Committee on Human Research and the Biosafety Committee of the University of California, San Francisco (Appendix A).

Phase 3: Cross reactivity of disinfectants

To determine if the reagent strip was cross reactive with common dental disinfectants, glass surfaces were measured after single and multiple applications of a disinfectant. A dilution of an iodophor disinfectant (Biocide) was mixed to the correct dilution (18cc to one gallon) with distilled water. A 1:10 dilution of sodium hypochlorite (Clorox) was mixed with distilled water. The disinfectants were mixed fresh for each day of the experiment.

For determining cross reactivity of the disinfectant with the reagent strips after a single application of the disinfectant, glass slides were sprayed once with the disinfectant. The slides were briefly tilted to allow excess solution to run off. Several slides were measured with the reagent strip immediately after application of the disinfectant. The disinfectant was allowed to dry for the remaining glass slides. The slides were sampled with the reagent strip every hour up to five hours. The slides were sampled by the technique developed for the preliminary studies. The slides were allowed to dry overnight and were

measured once following overnight drying.

For multiple applications of the disinfectant, the iodophor disinfectant was applied to glass slides for every hour until the disinfectant was applied five times. After each application, the slides were briefly tilted to allow excess solution to run off. Before each application, the slides were sampled with the reagent strip. The slides were allowed to dry overnight and measured after each application.

A stock solution of iodophor and sodium hypochlorite (5%) was applied to a glass slide. The slide was tilted to allow excess solution to run off of the slide. The slide was sampled immediately after application of the disinfectant and following overnight drying. Distilled water and a glass slide served as a negative control.

Phase 4: Removal of hemoglobin peroxidase activity with disinfectants

To determine the efficacy of a disinfectant to remove whole human blood from various surfaces, blood was applied to a surface and treated with the disinfectant. Again appropriate research consent was obtained to conduct this phase (Appendix A). 0.2 ml of whole human blood was applied to glass slides and 2 x 2" squares of naugahyde, formica and stainless steel. The unit of blood was obtained from Irwin Memorial Blood Bank. The blood was saturated with

anticoagulants. A clean cotton swab saturated with the blood was applied to the surface and the blood was spread in a circular motion on the surface. The surfaces were allowed to dry not less than 10 minutes. The surfaces were treated to one of two treatments. For one treatment the surfaces were sprayed with an iodophor disinfectant, or distilled water. A fresh dilution of an iodophor disinfectant (Biocide) was mixed to the correct dilution (18cc to one gallon) for every day of the experiment with distilled water. The surface was wiped with a clean gauze wipe. The other treatment consisted of spraying the surface with an iodophor disinfectant or distilled water and wiped with a clean gauze wipe. The surfaces received another spray of disinfectant and were wiped again with a new clean gauze wipe. After the treatment, the surfaces were tested with a reagent strip. Whole human blood applied to the glass slide, formica, naugahyde and stainless steel served as positive controls. A clean glass slide, formica, naugahyde, stainless steel square, and distilled water served as negative controls.

Phase 5: Scanning electron micrographs

0.2 ml of whole human blood was applied to a 2 x 2" square of stainless steel. A scanning electron micrograph

(SX-40A SEM, 15kV, 250-1000X)[1] was taken of the surface. No surface preparation was necessary prior to taking the SEM. The surface was either sprayed once with an iodophor disinfectant and wiped with a clean gauze wipe or received the same treatment twice prior to taking the scanning electron micrograph.

Statistical analysis

Frequency analysis for the second phase of the study of the ten surfaces in the dental operatories revealed that the data did not follow a normal (Gaussian) distribution. Consequently, non-parametric measures were utilized to analyze the data. The data was analyzed using a logistical analysis for ordinal data. A p value was obtained following the analysis of variance.

The low values, 0-3, were combined to equal a negative reaction and the high values, 4-5, were combined to equal a positive reaction. The data was analyzed using Chi-square and a p value was obtained. The level of significance was tested at $p \leq 0.05$.

For the surface sampling in the four university-based clinics, the amount of blood recorded as a range of values

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[1]CFAS technique
Department of Restorative Dentistry
University of California, San Francisco

represented the dependent variable. The independent variables was the time that the samples were taken and the surfaces and clinics which were sampled.

The independent variables for phase 4 of the experiment were the removal agents and the methods of removal of HPA.

III RESULTS

Phase 1: Testing the sensitivity of hemoglobin reagent strips and developing a sampling technique

Phase 1 of the experiment demonstrated that the reagent strips are consistently sensitive to dilutions of 10^{-5}. The values of the reagent strips when placed into serial dilutions of blood are shown in Table 11.

Table 11: Values for reagent strip from dilutions of whole human blood in sterile saline.

	1人 slides	5人 slides	1人 tubes	5人 tubes
no dilution	5	5	5	5
10^{-3}	5	5	4	5
10^{-4}	1	4	1	1
10^{-5}	1	1	1	1
10^{-6}	0	1	1	1

The sensitivity assessments of the reagent strips revealed the strips were very sensitive to the presence of hemoglobin peroxidase activity. The sensitivity is consistent regardless whether the strip was dipped into a diluted solution or if the solution was placed onto a glass slide. A surface assessment technique was developed in which the strip is moistened and place directly onto the surface of interest was developed. Table 12 shows that eight out of nine surfaces which were exposed to the patient's oral fluids of a patient were positive.

Table 12: Values for surface sampling of various surfaces by a reagent strip in three dental operatories.

Surface	Procedure	Values
bur	restorative	1
suction	restorative	0
angle	prophylaxis	5
water syringe	prophylaxis	4
faucet handle	prophylaxis	1
handpiece	operative	2
faucet handle	operative	1
water syringe	operative	3
composite dispenser	operative	1

Phase 2: Surface testing

In phase 2 of the experiment in which reagent strips were used to measure HPA activity on various surfaces in many dental operatories, a total of 795 surfaces were sampled. 397 surfaces were sampled at time 1, before patient treatment, and 395 surfaces at time 2, after patient treatment. There was a significant difference ($p \leq .01$) between the different surfaces and the values of the reagent strips at time 1. There was a significant difference ($p \leq .01$) between the surfaces and the values of the reagent strip at time 2. Table 13 demonstrates the positive and negative values for all of the measured surfaces time 1, before patient treatment. Table 14 demonstrates the positive and negative values for all surfaces at time 2, after patient treatment. Tables 15 and 16 represent the percent positive values for the ten surfaces at time 1 and time 2. For a 95% confidence level each value is $\pm$ 7.5 on either side of each value.

Table 13: **Positive and negative values for all surfaces before patient treatment.**

	positive value	negative value
Surface 1	22	18
Surface 2	10	27
Surface 3	39	1
Surface 4	35	5
Surface 5	33	7
Surface 6	19	21
Surface 7	8	32
Surface 8	16	24
Surface 9	22	18
Surface 10	70	12

Table 15: Percent positive values for the ten surfaces measured in the dental operatories before patient treatment.

	% Positive
Surface 1	45
Surface 2	73
Surface 3	3
Surface 4	13
Surface 5	17
Surface 6	53
Surface 7	80
Surface 8	60
Surface 9	45
Surface 10	30
Total	42

Table 16: Percent positive values for the ten surfaces measured in the dental operatories after patient treatment.

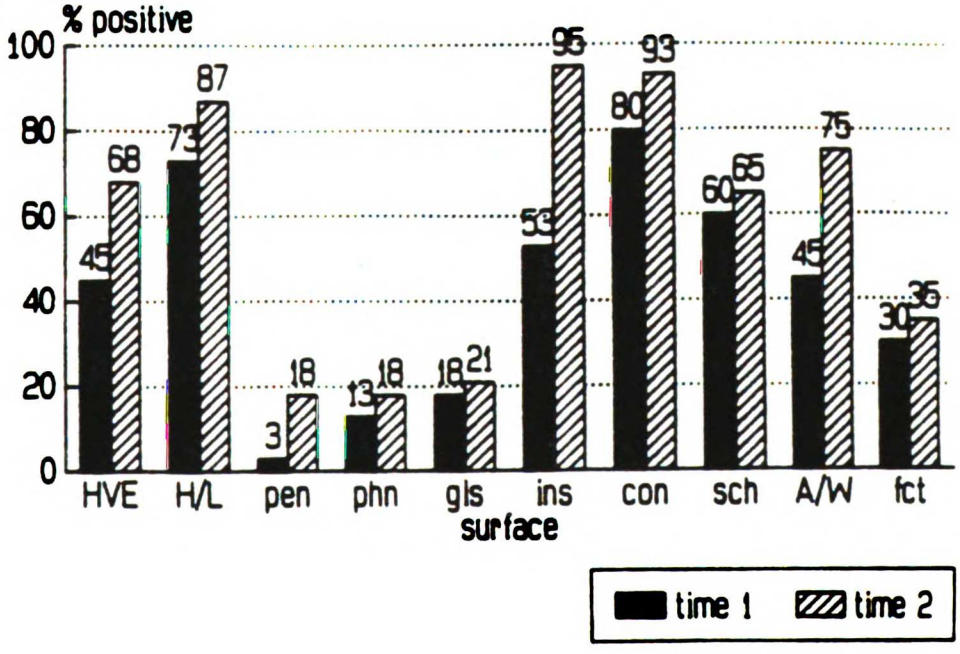
	% Positive
Surface 1	67
Surface 2	87
Surface 3	18
Surface 4	18
Surface 5	21
Surface 6	95
Surface 7	93
Surface 8	65
Surface 9	75
Surface 10	35
Total	57

There was a significant difference comparing all surface values for all clinics at time 1 and time 2. After patient treatment, there is an increase in the percent of HPA activity for all surfaces. At time 1, the total percent

positive for HPA activity is forty two percent. At time 2, the total percent positive for HPA activity is fifty seven percent. 392 out of 794 of all surfaces, regardless of time of sample, tested positive.

A comparison of the each surface (percent positive) for all clinics at time 1 and time 2 revealed that surfaces such as the pen, telephone, faucet handle and safety glasses had low levels of activity (≤35%) regardless of time of sample compared to the high volume evacuation, the handpiece, an instrument, the connector to the handpiece, the air/water syringe, and the master switch (≥65%). Figure 5 depicts a histogram which compares the values of the reagent strips at time 1 and time 2.

Figure 5: Values (% positive) for surfaces sampled before and after patient treatment.



There was no significant difference ($p \leq .39$, chi-square) between the four different clinics measured and the amount of activity at time 1, prior to patient treatment.

At time 2, immediately following patient treatment, there was a significant difference ($p \leq .01$, chi-square) between the four clinics measured and the amount of HPA activity. Table 17 presents the percent positive values of the sampled surfaces in each clinic at time 1. Table 18 shows the percent positive values for the four clinics measured at time 2.

Table 17: Percent positive surfaces for the four clinics measured before patient treatment.

	% Positive
Clinic 1	47
Clinic 2	38
Clinic 3	44.4
Clinic 4	37
Total	42

Table 18: Percent positive surfaces for the four clinics measured after patient treatment.

	% Positive
Clinic 1	77
Clinic 2	42
Clinic 3	64
Clinic 4	48
Total	58

Regardless of time of sample, clinics 2 and 4 demonstrated less HPA activity than clinics 1 and 3. The clinic demonstrating the highest percentage of HPA activity was the predoctoral clinic followed by the periodontal surgical clinic, the faculty group practice and the oral surgery clinic.

The data were combined to compare the ten surfaces for all four clinics at time 1 and time 2. Table 19 is the positive percent values for the ten sampled surfaces at time 1. Table 20 is the positive percent values for the ten sampled surfaces at time 2.

Table 19: Percent positive values for surfaces and clinics before patient treatment.

		CLINIC				
		1	2	3	4	Surface Mean *
SURFACE						*
HVE on/off	(HVE)	60	30	40	50	45
H/L handpiece	(H/L)	70	50	89	88	74
pen/pencil	(pen)	0	10	0	0	3
telephone	(phn)	10	10	30	0	13
safety glasses	(gls)	10	20	40	0	18
instrument	(ins)	60	20	40	90	53
H/L connector	(con)	90	90	70	70	80
master switch	(sch)	80	50	50	60	60
A/W syringe	(A/W)	70	60	50	0	45
faucet	(fct)	20	40	40	20	30
clinic mean for all surfaces * * * * *		47	38	44	37	42

Table 20: Percent positive values for surfaces and clinics after patient treatment.

		Clinic				
		1	2	3	4	Surface Mean ***
SURFACE						
HVE on/off	(HVE)	90	50	70	60	68
H/L handpiece	(H/L)	100	50	100	100	88
pen/pencil	(pen)	40	0	20	10	18
telephone	(phn)	30	20	11	10	18
safety glasses	(gls)	44	0	33	10	22
instrument	(ins)	100	80	100	100	95
H/L connector	(con)	100	90	90	90	93
master switch	(sch)	100	30	60	70	65
A/W syringe	(A/W)	100	90	100	10	75
faucet	(fct)	50	10	50	30	35
clinic mean for all surfaces * * * *		76	42	64	48	58

Phase 3: Cross reactivity of disinfectants

Phase 3 investigated cross reactivity of the reagent strips with 5% sodium hypochlorite (1:10, 1:100) and against an iodophor disinfectant.

To determine if the disinfectants gave different values for the reagent strip over time, glass slides were treated with a single application of an iodophor disinfectant or a 1:10 dilution of sodium hypochlorite. Tables 21 and 22 demonstrate the reagent strip values for glass slides treated with a single application of one of the disinfectants. The surfaces were measured every hour up to five hours.

Table 21: Values for glass slides treated with a single spray of an iodophor disinfectant and measured every hour up to five hours.

	slide 1	slide 2	slide 3
0 hour	0	0	0
1 hour	0	0	0
2 hours	0	1	0
3 hours	0	1	1
4 hours	0	1	0
5 hours	1	1	0

Table 22: Values for glass slides treated with a single spray of an 1:10 dilution of sodium hypochlorite and measured every hour up to five hours.

	slide 1	slide 2	slide 3
0 hour	4	5	5
1 hour	1	4	5
2 hours	0	0	4
3 hours	0	0	0
4 hours	0	0	0
5 hours	0	0	0

Surfaces which were treated with sodium hypochlorite gave high values for the reagent strips, however, after 3 hours negative values were obtained. Surfaces treated with an iodophor disinfectant gave low values (0-2) regardless of time of sample. Subsequently, the data from the surface sampling of the dental operatories was collapsed to account for the cross reactivity. All samples which gave values of zero to three were collapsed and assumed to be a negative value. Samples which gave values greater than three were assumed to be a positive value. Higher dilutions of an iodophor disinfectant also gave higher values.

To determine if the disinfectants gave different values for the reagent strip from multiple applications of a disinfectant, glass slides were treated with multiple applications an iodophor disinfectant or a 1:10 dilution of sodium hypochlorite, allowed to dry between applications and measured with a reagent strip (Table 23, 24). The slides were also measured after overnight drying (Table 25, 26).

Table 23: Values for glass slides which received five applications of an iodophor disinfectant and allowed to dry for one hour between applications.

hour	1	2	3	4	5
value	0	0	0	0	0

Table 24: Values for glass slides which received five applications of a 1:10 dilution of sodium hypochlorite and allowed to dry for one hour between applications.

hour	1	2	3	4	5
value	4	4	2	5	2

Table 25: Values for glass slides which received a single application of an iodophor disinfectant, allowed to dry for one hour between treatments, and received another application for every hour up to five hours. Slides were measured after over night drying.

hour	22	23	24	25	26
value	0,0,0,0	0	0	0	1

Table 26: Values for glass slides which received a single application of a 1:10 dilution of sodium hypochlorite, allowed to dry for one hour between treatments, and received another application. Slides were measured after over night drying.

hour	22	23	24	25	26
value	1,1,0,0	1	1	1	0

Because iodophor disinfectants and sodium hypochlorite are diluted solutions, stock solutions were measured for cross reactivity. Table 27 represent the reagent strip values for the glass slides treated with a single application of stock solution of the iodophor and sodium hypochlorite solutions.

Table 27: Stock solutions of a iodophor disinfectant and a 1:10 sodium hypochlorite solution were applied to glass slides. Slides were allowed to dry over night and measured.

	0 hour	26 hours
iodophor	5+	5
bleach	4	0

Phase 4: Removal of hemoglobin peroxidase activity with disinfectants

The efficacy of an iodophor disinfectant, distilled water and alcohol in removing whole human blood from the counter-top material (Formica), vinyl cloth (Naugahyde) and stainless steel squares revealed that HPA was present on the surface regardless of the two methods of treatment to remove the blood. All surfaces gave high values (4-5) for the reagent strips. None of the surfaces which experienced the treatments gave negative or even low values. The values for the surfaces treated with a single spray and wipe of distilled water are recorded on Table 28. The surfaces treated with a single spray and wipe of an iodophor disinfectant gave similar results. The values for surfaces which were sprayed and wiped twice also gave similar results with no value less than four.

Table 28: 0.2 ml of blood was applied to squares of stainless steel, counter-top material and vinyl cloth; treated with a single spray of distilled water; wiped with a clean gauze wipe; and measured.

	stainless steel	counter-top	vinyl cloth
Surface 1	5	5	5
Surface 2	5	5	5
Surface 3	5	5	5
Surface 4	5	5	5
Surface 5	5	5	5
Surface 6	5	5	5
Surface 7	5	5	5
Surface 8	5	5	5
Surface 9	5	5	5
Surface 10	5	5	5

0.05 ml of blood was applied to 2 x 2" squares of glass, counter-top material and vinyl cloth. These surfaces were treated with a spray of the iodophor disinfectant and wiped with a clean gauze. The surface was sprayed and wiped again. Results of using the iodophor disinfectant to remove the blood from the surface is recorded in Table 29.

Table 29: Values for the reagent strip when 0.05 ml of blood is placed onto squares of counter-top material, glass, and vinyl cloth; treated with a spray of an iodophor disinfectant; wiped; sprayed; and wiped again.

	stainless steel	counter-top	vinyl cloth
Surface 1	3	3	5
Surface 2	3	2	5
Surface 3	4	4	5
Surface 4	5	4	5
Surface 5	5	3	5

The iodophor disinfectant and the 1:10 sodium hypochlorite solution removed visible signs of blood on most smooth surfaces, however 70% alcohol removed very little or none of the blood.

It was demonstrated in phase 4 of the experiment that it was difficult to remove blood placed on different surfaces with various agents. HPA activity was measured at high values (4-5) regardless of the various treatments. Although the surfaces were sprayed, wiped, sprayed and wiped again, the reagent strips still gave high values. It was clearly demonstrated in this phase of the experiment why previously measured surfaces had substantial levels of HPA regardless of time of sample. As was demonstrated by Molinari et al, surfaces treated with alcohol failed to removed the bulk of the blood[79-80].

Figures 6, 7, and 8 are scanning electron micrographs were taken of a stainless steel surface.

Figure 6: Scanning electron micrograph of whole human blood placed on a stainless steel surface.

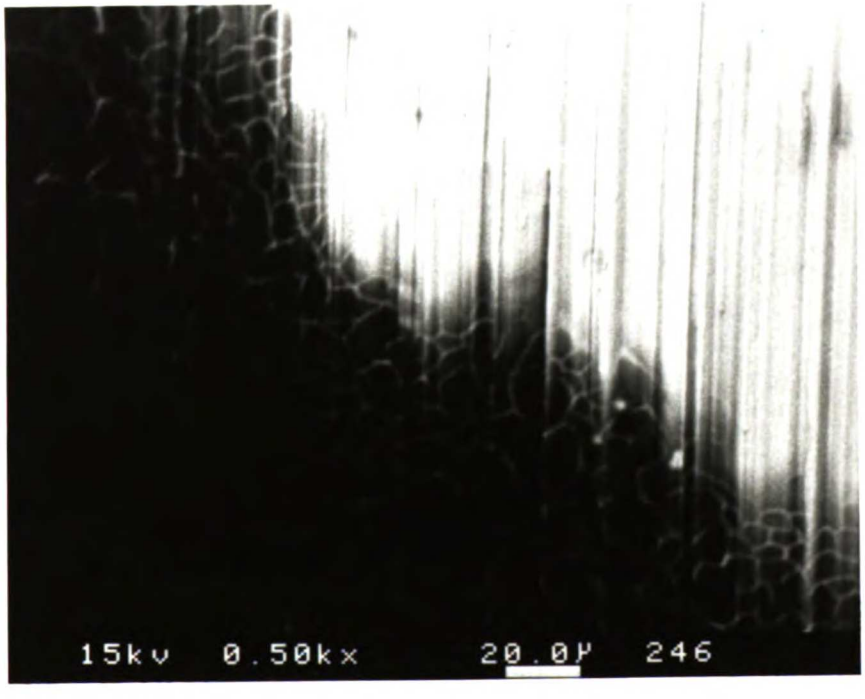


Figure 7: Scanning electron micrograph of whole human blood placed on a stainless steel surface, sprayed with an iodophor disinfectant and wiped with a clean gauze wipe.

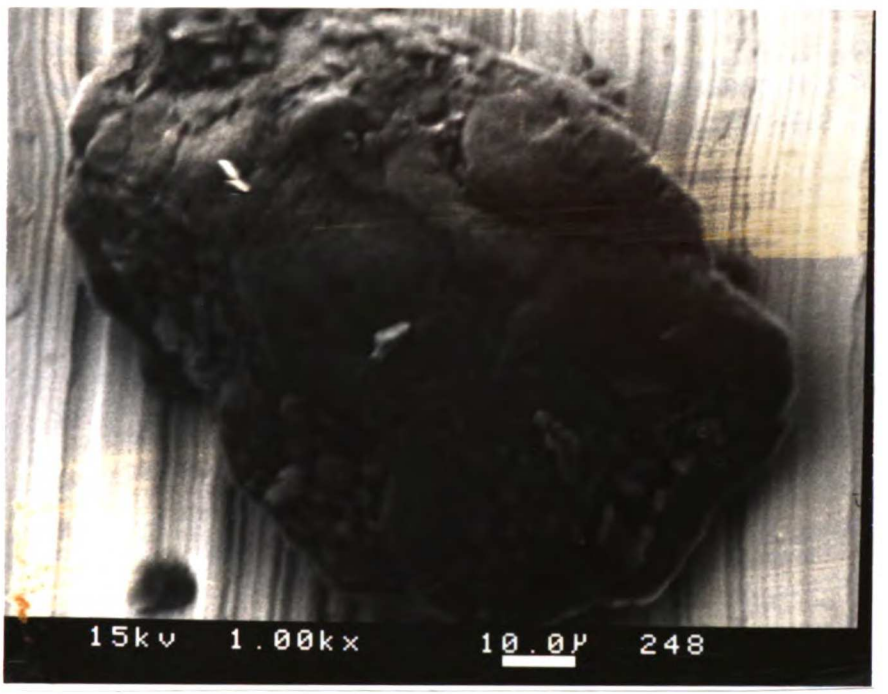
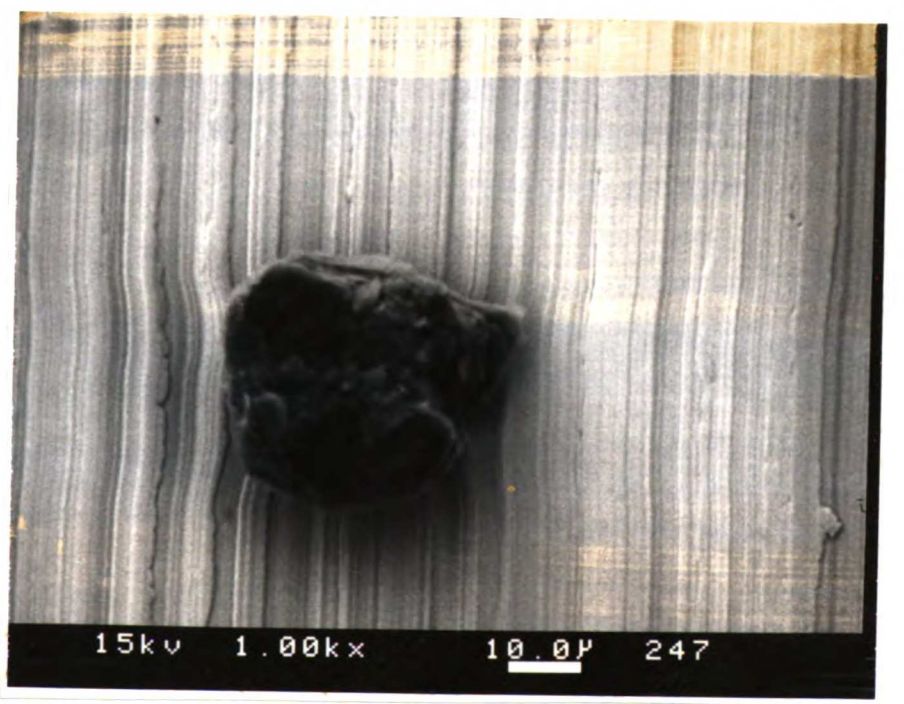


Figure 8: Scanning electron micrograph of whole human blood placed on a stainless steel surface, sprayed with an iodophor disinfectant, wiped, sprayed, and wiped again.



Summary of findings

The first phase of the study demonstrated that the reagent strips are sensitive to 10^{-5} dilutions of blood and inanimate surfaces can be sampled by pressing a moistened reagent strip directly onto the surface.

The second phase of the study looked at ten specific surfaces of clinical operatories in a university-based dental school. Surfaces such as the air/water syringe, the high speed connector, the sterile instrument, the handpieces and the high volume evacuation all gave relatively high values of HPA activity ($\geq$ 45%). Surfaces such as the pen/pencils, the telephone, the safety glasses, and the faucet handle gave relatively low values of activity ($\leq$ 35%). Surfaces which demonstrated high levels of HPA (>50%) before patient treatment remained at high levels after patient treatment. Surfaces which demonstrated low levels of HPA before patient treatment remained at low levels after patient treatment. As was expected, all surfaces experienced an increased percentage of HPA activity after patient treatment. All surfaces revealed HPA activity, suggesting that hemoglobin may have been present in the hemolyzed form. Each surface demonstrated different levels of activity in different clinics.

Because some of the surfaces sampled in the second phase of the study were routinely decontaminated, the next phase of the study was to determine possible cross

reactivity of the strips with common dental disinfectants (1:10 sodium hypochlorite and iodophor). Both disinfectants were utilized in the clinics sampled at phase 2. Iodophor disinfectants reacted with the reagent strips to give low positive values (0-3). However, higher values were not obtained. Low positive values from the surfaces in the clinics sampled in phase 2 of the study could be reactions from the iodophor disinfectant utilized in the clinic. To account for this, low values (0-3) obtained in phase 2 of the study were combined and considered to be a negative value (0). Values higher than three were considered to be a positive value. If a surface was treated with a 1:10 dilution of sodium hypochlorite and measured with a reagent strip, in less than three hours the strip yielded high positive values (4-5). After three hours, surfaces treated with sodium hypochlorite yielded negative values. For the surfaces sampled in phase 2 of the study, at least 3 hours had been allowed before the surface was measured.

The final phase of the study was to determine if various solutions could remove dried blood from a surface. Whole human blood was applied to three different surfaces, allowed to dry, and treated with various solutions. The effectiveness of an iodophor disinfectant, isopropyl alcohol and distilled water in removing dried blood from common dental surfaces was assessed with reagent strips. Isopropyl alcohol (70%) removed little or none of the dried blood.

The iodophor disinfectant and distilled water removed visible signs of blood applied to various surfaces; however, the solutions failed to decrease HPA activity. Scanning electron micrographs revealed organic debris present on surfaces which had been treated with an iodophor disinfectant. The micrographs revealed slightly distorted biconcave forms, approximately 7 μm in diameter, imbedded in a non-distinct material. This shape and size suggest that these biconcave forms represent intact red blood cells.

IV CONCLUSION

Because dental health care workers have unavoidable contact with the oral fluids of patients, it is important to develop surface assessments to determine the dispersal of potentially hazardous fluids within the dental environment. Appropriate surface assessments can determine which surfaces or items require decontamination or sterilization. Surface assessments can also monitor the infection control practices of the dental staff because oral fluids can be transferred by the gloved or ungloved hand of practitioners.

Previous studies have used reagent strips sensitive to the peroxidase-like activity of hemoglobin for assessing the presence of blood on surfaces in occupational settings where blood or blood products are handled. In the present study, a surface assessment was utilized for environmental surfaces in dentistry.

The assessment used in this study can determine which surfaces or items in the dental suite require sterilization or decontamination. Furthermore, the reagent strip technique is a reliable assessment which can guide infection control protocols and policy decisions. For example, our results would suggest that because it is so difficult to remove dried blood from a surface, oral fluid contamination of instruments and equipment which cannot be sterilized should be minimized. This is especially true for instruments or equipment which have corrugated or rough surfaces such as the on/off switch to the high volume evacuator and the high speed connector. Our results are supportive of the Centers for Disease Control recommendations that surfaces which may be exposed to blood and blood products should be covered with an impervious material.[86]

The surface assessment implemented in our study can monitor the infection control practices of staff members. Surfaces which are not normally touched during the course of treatment should have negative HPA values. Because reagent strips give immediate results (within forty seconds), the feedback of the reagent strip results may offer reinforcement for behavioral changes of dental personnel.

Perhaps the most appropriate use for the reagent strips is an evaluation of aseptic techniques for equipment and surfaces which are not routinely disinfected such as the

telephone. Our results demonstrated that the lowest percentage of positive surfaces was in the oral surgery clinic. This suggests that the dispersal of the patients' oral fluids can be minimized even when there is predictable contact with blood.

The difficulty in removing dried blood from a surface raises some concern about the environmental hazards of surfaces in the dental operatory; however, it was not possible to assess the actual risk of infection from a surface which demonstrated positive values for the reagent strips. Further studies will be needed to determine if reagent strips are a reasonable assessment of infectious disease transmission from inanimate surfaces in the dental operatory. If the strips are an appropriate environmental assessment, then cross reactivity with other common dental disinfectants (other than the ones tested in this study) will be needed.

Because the reagent strips are limited to identifying the presence of a potentially hazardous material, quantitative studies will be needed to determine the risk of disease transmission and the presence of organic matter or bioburden on inanimate surface. If bioburden is associated with disease transmission, then techniques will need to be developed to facilitate the removal of blood and other organic debris.

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APPENDIX A

SAN FRANCISCO: SCHOOL OF DENTISTRY
DEPARTMENT OF DENTAL
PUBLIC HEALTH AND HYGIENE

To: Barry Engelstad, MD
Chairman
Committee on Human Research

From: Cara M. Miyasaki
Graduate student in Oral Biology

RE: The detection of blood on environmental surfaces in a university-based dental school; an assessment of infection control practices.

Attached is a request for adminstrative review of the project named above. I have also attached a short form face sheet with the signature of the Principal Investigator, who is my sponsor for the project.

Thank you for your attention to this matter.

Cara M Miyasaki

ool of Dentistry
artment of Dental Public
ealth and Hygiene
Francisco, CA 94143

To whom it may concern:

The application for the study: The detection of blood on environmental surfaces in a university-based dental school; an assessment of infection control practices will be a study conducted toward completion of a Master's of Science degree in Oral Biology. The attached short form application has partial completion of pertinent information. We wish to claim exempt status for the study.

Thank you,

Cara M Miyasaki

Cara M. Miyasaki

COMMITTEE ON HUMAN RESEARCH
INITIAL SHORT-FORM APPLICATION

Principal Investigator & Degree (UCSF Faculty) John S. Greenspan | **University Title** Prof & Chair | **Dept.** Oral Biology

Mailing Address (Campus) HSW 604 Box 0512 | **Phone Number** 62220, 62943 | **Is P.I. sponsor/ advisor only?** yes

Co-Investigator Cara M. Miyasaki | **Title** GraduateMSstudent | **Dept.** Oral Biology

Mailing Address School of Dentistry Deptof DentPub Health&Hyg | **Phone Number** 69879, 69889 | **Submission Date** 8/23/88

Project Title The detection of blood on environmental surfaces in a university-based dental school an assessment of infection control practices.

INSTRUCTIONS: Please type. The following should be submitted:
- -1 COPY OF THE HEPC COVER SHEET
- -5 COPIES OF THIS COMPLETED SHORT-FORM
- -5 COPIES OF ALL CONSENT FORMS/INFORMATION SHEETS
- -5 COPIES OF ALL SPECIAL REQUIREMENTS/ATTACHMENTS

NOTE: See PART V of the UCSF GUIDELINES FOR RESEARCH INVOLVING HUMAN SUBJECTS (10/87) for further information and directions on all of the above. Please allow approximately 4-6 weeks for the CHR review process to be completed.

1. STUDY AIM, BACKGROUND AND DESIGN

2. SUBJECT POPULATION: INCLUSION/EXCLUSION CRITERIA, USE OF SPECIAL SUBJECT GROUPS, AND METHODS OF ACCESS

3. PROCEDURES TO BE DONE FOR PURPOSES OF THE STUDY

4. **RISKS: POTENTIAL RISKS/DISCOMFORTS TO SUBJECTS AND METHODS OF MINIMIZING THESE RISKS**

5. **BENEFITS: POTENTIAL DIRECT BENEFITS TO SUBJECTS AND GENERAL BENEFITS TO SUBJECT GROUP, MEDICAL SCIENCE AND/OR SOCIETY**

6. **CONSENT PROCESS AND DOCUMENTATION**

7. **NUMBER OF SUBJECTS TO BE ENROLLED PER YEAR:___**
TOTAL FOR STUDY:____

8. **WILL THIS STUDY BE FUNDED? Yes___ No___ Pending___**
AGENCY/SPONSOR?_____

9. **THE EXPEDITED REVIEW CATEGORY NUMBER IS ___ (from PART V-B of the UCSF GUIDELINES FOR RESEARCH INVOLVING HUMAN SUBJECTS, 10/87).**

10. **PRINCIPAL INVESTIGATOR'S SIGNATURE**_____

EXPERIMENTAL PROTOCOL:

The sampling procedure will involve the direct sampling of chosen surfaces with a reagent strip. The reagent strip detects trace amounts of hemoglobin. The reagent strip will be moistened with sterile saline and placed on the surface of interest. The color of the strip is read after forty seconds. The contaminated strips will be placed in autoclavable biosafety containers, sealed, and processed in an appropriate manner.

COMMITTEE ON HUMAN RESEARCH
ADMINISTRATIVE REVIEW FORM (TO CLAIM EXEMPT STATUS)

Investigator John S. Greenspan BDS, Phd, FRC Path **Address** HSW 604 **Phone** 62220, 62943

Study Title The detection of blood on environmental surfaces in a university-based dental school: an assessment of infection control practices. **Submission Date** 8/23/88

1. **Give a brief description of this study.**
   The purpose of this study is to detect trace amounts of blood on various inanimate surfaces. The sampling will involve the swabbing of various surfaces during various times of the day and before and after sterilization and/or disinfection procedures. The surfaces will be identified by numbers. The dental school clinics will be: Oral Medicine, Periodontal Surgery, Oral Surgery, Predoctoral, Faculty Practice.
2. **Which exempt category does this research fall within?** # 5 (From PART VIII-B, UCSF GUIDELINES FOR RESEARCH INVOLVING HUMAN SUBJECTS, October, 1987)
3. **Will any subjects be contacted for purposes of the study?** No X Yes ___
   If so, please describe the contact process.

   The collection of data will only involve sampling of surfaces in several dental clinics within University of California, San Francisco, School of Dentistry.

4. **Will subjects be completely anonymous?** Yes X No ___
   If not, how will their identities be coded? How long and in what way will records be retained? Who will have access to study data?

   The collection of data will only involve the identification of the surface which will be sampled.

5. **Will there be any data collection sheets used for study purposes?** No X Yes ___
   If so, please submit one copy of each. The denal clinicians will not be identified. The surfaces which will be sampled will be identified by numbers.

CERTIFICATION OF EXEMPT STATUS

On the basis of the information presented here, this research activity qualifies as exempt from review by the Committee on Human Research.

[signature] 8/23/88

Principal Analyst, Committee on Human Research — Date
Human & Environmental Protection Committees
Office of Research Affairs, UCSF

DENIAL OF EXEMPT STATUS

On the basis of the information presented here, this study does not qualify for exempt status, and an application should be submitted to the Committee on Human Research for review by the:

___ SUBCOMMITTEE (for Expedited Review)
___ FULL COMMITTEE

Chairman, Committee on Human Research — Date

10/87

Log #_____

UCSF BIOSAFETY COMMITTEE
INFECTIOUS AGENTS APPLICATION - COVER SHEET

This form is for work involving Infectious Agents. (See listing in Appendix A of the Biosafety Guidelines.) Submit 1 copies of your application to Box 0616

PROJECT TITLE The detection of blood on environmental surfaces in a university-based dental school; an assessment of infection control practices

NAME OF INFECTIOUS AGENT blood, tooth structure, gingival exudate, saliva

Principal Investigator John S. Greenspan **Faculty Title & Dept.** Prof&Chm Stomatology

Mailing Address HSW 604 **Phone #** 62220, 62943

Co/other Investigators (optional) Cara M. Miyasaki

GRANT APPROVAL NUMBER_____ **GRANT APPROVAL DATE**_____

Please complete the information below in detail. Any omissions may cause delay of the approval.

1. AGENT DESCRIPTION

Attach a brief summary description of the agent, its usual or possible host and vector(s), transmissibility to man and animals, and pathogenicity. What are the implications of possible release of the organism to the environment?

2. PROTOCOL

Attach a description of the protocol, including general experimental design, laboratory safety precautions, containment, decontamination and disposal, procedures for spills, and medical monitoring of personnel.

3. ANIMALS

Will animals be used? Yes____ **No** X **Species**_____

(If animals are used, approval from Committee on Animal Research is also required.)

Attach a description of the personnel protective measures; animal care and housing precautions; procedures for infecting, handling, and transporting animals, and for containment, decontamination and disposal of infected animals and waste. (A copy of this protocol shall be posted in the animal care facility if it is to be used.) Where will animals be infected and where will they be housed once infected?

4. LOCATION OF WORK WITH INFECTIOUS AGENT

Room(s): HSW604 D3237 _____

Classification P2* P2 _____

(Is room certified? yes **Attach a copy of the Inspection & Certification Form)**

5. PERSONNEL INVOLVED(list names)

Cara M. Miyasaki

Joel White

_____

_____

Office Use:

Approval Number_____

Approval Date _____

Expiration Date_____

1/24/89 _____
Date **Signature, Principal Investigator**

_____ _____
Date **Signature, Chairman, Biosafety Committee**

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
HUMAN AND ENVIRONMENTAL PROTECTION COMMITTEES
BOX 0616

HEPC COVER SHEET

PLEASE LIST ALL HEPC REVIEW COMMITTEES TO WHICH THIS PROTOCOL HAS BEEN OR WILL BE SUBMITTED. INCLUDE PERSON LISTED AS PRINCIPAL INVESTIGATOR, APPLICATION TITLE, DATE OF SUBMISSION, AND APPROVAL NUMBER (IF ISSUED). (Whenever possible, the same P.I. and study title should be used on each).

Committee	Principal Investigator	Application Title	Date of Submission	Approval # (if issued)
Committee on Human Research				
Committee on Animal Research				
Radiation Safety Committee				
Radioactive Drug Research Committee				
Biosafety Committee	JohnS.Greenspan	The detection of blood on environmental surfaces in a university-based dental school; an assessment of infection control practices	1/24/89	

UCSF BIOSAFETY COMMITTEE
INFECTIOUS AGENTS APPLICATION

1. AGENT DESCRIPTION

The health care worker will be exposed to surfaces possibly contaminated with saliva, blood, gingival exudate and tooth structure. The amount of each is undetermined but it will be less than or equal to the amount in which a dentist or dental hygienist is exposed to during one treatment procedure. These fluids will not be aerosolized by rotary instruments or contacted with ungloved hands.

These surfaces may contain a variety of infectious organisms including herpes simplex, hepatitis and the human immunodeficiency syndrome. It has been demonstrated that blood containing herpes simplex, hepatitis and the human immunodeficiency virus can be infectious if introduced by a percutaneous route via needlestick or puncture with a sharp instrument. Herpes, hepatitis and HIV can be infectious if the virus contacts mucous membranes or non intact skin.

Infection of mucous membranes or skin (ie herpetic whitlow) with herpes simplex usually produces a local infection which is self-limiting in healthy individuals. Symptoms of primary herpes infection may range from mild to serious. Serious infections may lead to multiple ulcerations. If the infection is oral, the individual may experience difficulty chewing, limited opening and pain. If the infection is located on the skin such as a finger, the individual may experience swelling, ulcerations, tenderness and pain.

HIV is a fragile organism and cannot live outside the host for very long. Inadvertent contamination of the laboratory environment with the organism may cause infection of those individuals in contact with the organisms.

2. PROTOCOL

a. Not more than ten inanimate surfaces in a dental operatory room will be sampled with a reagent strip which detects the peroxidase-like activity of hemoglobin. The strip is moistened with sterile saline and placed on the surface of interest. The color of the strip is read after forty seconds.

b. After the patient receives the dental treatment, the surfaces will be sampled again prior to disinfection of the surfaces. Reagent strips will be used as described above.

c. The contaminated strips will be placed in an autoclavable biosafety container, sealed and autoclaved for fifteen minutes, 250-275°F.

e. All contaminated disposable items such as gloves and gowns will be placed into a double-bagged biosafety container, sealed and autoclaved for fifteen minutes, 250-275°F.

f. The dental operatory will be disinfected with the appropriate disinfectants in preparation for the next patient.

The study investigator will follow university biosafety precautions. Latex gloves will be worn during the sampling of surfaces. Contaminated waste will be immediately placed in an impervious biosafety container, sealed and disposed of in an appropriate manner.

Accidental spills of possibly infectious agents will be properly decontaminated. The area will be flooded with a 1:10 dilution of sodium hypochlorite and left for at least fifteen minutes. The area will be wiped with absorbent paper towels which will be placed in an impervious biosafety container and autoclaved.

In the case of inadvertent splash to mucous membranes or percutaneous exposure, the laboratory technician will be evaluated serologically for evidence of HIV and HBV infection as soon as possible following the exposure. If any acute febrile illness occurs within 12 weeks after exposure, the technician will be advised to report and seek medical evaluation. A lab technician which tests negative for HIV will be retested in six weeks post-exposure, twelve weeks and at six months.

BIOSAFETY COMMITTEE
Division of Human & Environmental
Protection Committees, Box 0616
Office of Research Affairs
University of California, San Francisco

April 15, 1988

To: John S. Greenspan, B.D.S., Ph.D.
Box 0512
x2220

From: the Biosafety Committee

Re: **Status of your Biosafety approval # 1-063-01**

Title:
UCSF AIDS TISSUE AND SERUM BANK

In accordance with our responsibility to re-review approved Infectious Agents and Recombinant DNA applications at appropriate intervals, and in order to minimize paperwork and record-keeping, the Biosafety Committee is querying all Principal Investigators with BSC approval dates between 1980-1983 (see attached). Would you please take a few moments to complete this one page questionnaire? If you have any questions, call Kathy Wiemelt at 476-2198.

At your earliest convenience, please return this questionnaire to:
Biosafety Committee
Box 0616

___ This protocol is no longer active and there are no plans to re-activate this study in the future.

___ There is a more current approval that subsumes the above named study. The approval # of the more current study is _____

XX This protocol is currently active.
(attached)
XX An addendum includes a revised list of names for the personnel involved.
___ Any additional findings that would alter the level of containment? If so, then attach an addendum to explain.

Comments_____

(X) _____ 5/13/88
John S. Greenspan, B.D.S., Ph.D. date

Re-approved at the June 14, 1988 Biosafety Committee meeting.

Jeffrey Felton
Jeffrey Felton, Chairman, BSC

6/22/88
date

August 3, 1988

DR JOEL WHITE, DDS
RESTORATIVE DENTISTRY
SCHOOL OF DENTISTRY
BOX 0758 D-3247

Re: Laboratory Inspection

As per your request, your laboratory on the third floor of the Dental Building, room 3247 was inspected on the morning of August 3, 1988. The inspection reveal that you will be working with bacteria associated with flora normally isolated from the mouth. The UCSF Biosafety Committee classifies your work to require a P2 containment level.

Upon inspection of your facility, your laboratory conforms with present University and CDC/NIH Guidelines for the P2 Physical Containment Level. All standard microbiological practices shall be strictly enforced. This shall include, but not be limited, to the following:

(1) Eating, drinking, smoking and application of cosmetics in the laboratory are strictly prohibited.
(2) Lab coats are required in the P2 facility and shall not be worn outside the laboratory.
(3) Transport of infectious materials or waste materials for disposal shall be transported in labelled durable leak-proof container(s) with tightly closed lid(s). Autoclave tape shall be used.
(4) The use of an effective chemical disinfectant shall be used at the initiation & termination of each days work.
(5) Access to fire extinguishers, chemical & biological spill kits, and access to telephones are highly recommended in event of emergency situation.

For your records, I have enclosed a copy of (1) the laboratory inspection report, (2) biohazard stickers, (3) a P2 sign, (4) Guidelines for the Preparation of an Emergency Action Plan, and (5) an Index of Audio/Visual Safety Aids.

Laboratory personnel should take care to label and post signs/ stickers correctly. The P2 sign & biohazard stickers shall be displayed in areas where biohazards are present and in a manner that is visible to laboratory personnel, visitors, and students. The purpose of these signs and stickers are to warn all laboratory and non-laboratory personnel to the potential hazard while working in the laboratory. In addition, the needed emergency information will be used to contact individual should the need arise.

Alfred L. Jin
Biohazard Safety Officer

6-0918
6-3028

UCSF LABORATORY INSPECTION REPORT
LABORATORY CONTAINMENT LEVELS FOR BIOLOGICAL RESEARCH

Principal Investigator: DR JOEL WHITE, DDS RESTORATTVE DENTISTRY Bldg/Rm: D-3247

Biohazard: ORAL FLORA, CELL CULTURE Containment Level: P-2

Signed: [signature] Date: CERTIFICATION DATE: AUGUST 3, 1988

	P1	P2	P3	P4
A. Hazard Levels	Basic/Low Risk	Basic/Low To Moderate	Moderate To High Potential Lethal Consequences	High Risk
B. Standard Microbiological Practices				
1. Public Access While Experiments Are In Progress	Not Recommended	Not Permitted OK	Prohibited/ Controlled	Prohibited/Controlled
2. Daily Decontamination.	Daily & Upon Spills	Daily & Upon Spills OK	Daily, Upon Finished Work And Spills	Daily, Upon Finish Work And Spills
3. Infectious Waste Decontamination.	Before Disposal	Before Disposal OK	Before Disposal	Before Disposal
4. Pipetting.	Mechanical Devices	Mechanical Devices OK	Mechanical Devices	Mechanical Devices
5. Eating, Drinking, Smoking, and Application of Cosmetics.	Not Permitted	Not Permitted OK	Not Permitted	Not Permitted
6. Handwashing Facilities.	Required	Required OK	Required	Foot/Elbow Operations
7. Minimization of Aerosol Production	Recommended	Recommended OK	Recommended	Recommended
8. Laboratory Coats.	Recommended	Required, Not Worn Outside the Laboratory (Ie Front Button Coats) OK	Required, Not Worn Outside Laboratory (Ie Wrap Around Coat)	Outer Change Room & Shower Facilities Required.
C. Special Practices				
1. Autoclave On-Site Facility	Not Required	Not Required But Available Within Building OK	Required On-Site	Double Door Autoclave Chamber Required On-Site
2. Insect/ Rodent Control Program	Required	Required OK	Required	Required
3. Transport of Infectious Material or Waste Materials For Processing (Ie Decontamination) Away From the Lab	Durable Leakproof Container	Durable Leakproof Container OK	Durable Leakproof Container	Non-Breakable Sealed Labeled Primary and Secondary Containers via Disinfection Dunk Tank, Fumigation Chamber/Air Lock

CDC-NIH Summmary of Contain-

4. Animals not Involved with Lab Experiment(s)	Not Permitted	Not Permitted OK	Not Permitted	Not Permitted
D. Containment Equipment				
1. Biological Safety Cabinets or other physical containment system	Recommended	Required with Aerosol Generating Equipment, with use of Large Concentrations, Animal Inoculations & Harvesting Infected Tissues from Animals or Eggs. NA OK	Required with Aerosol Genera Equipment, Use of Large Concentrations, Inoculation of Animals, Harvesting Infected Tissues from Animals or Eggs and Necropsy of Infected Animals.	Required Use of a Cl: III Biological Safety Cabinet In Conjunctic With a Double HEPA Exhaust Ventilation S: Without recirulation c a Special Designed La
	Retesting Every 12 months	Retesting Every 12 Months NA OK	Retesting Every 12 Months	Retesting Every 6 Months
2. Personal Protective Equipment (ie Gloves, mask, surgical respirators & etc.)	Recommended	Required When Handling Infected Animals OK	Required When Handling Infected Animals	Required When Hand ling Infected Animals
E. Laboratory Facilities				
1. Ventilation	Negative Pressure	Negative Pressure Open Windows Requires Screens OK	Negative Pressure No Recirculation of Air to Other Areas of the Building	Negative Pressure Sen tive Pressure Alarmed Manometers. All Air E hausted thru HEPA Fil and Discharged Away f Occupied Buildings an Intake Vents
2. Posted Hazardous Sign	Recommended	Required OK	Required	Required
3. Bench Top Work	Permitted	Permitted OK	Not Permitted	Not Permitted
4. Opened Laboratory Windows	Permitted With Fly Screen	Permitted With Fly Screen OK	Not Permitted	Not Permitted
5. Laboratory Separated From the General Public	No	No OK	Yes	Yes
E. Training				
1. Technical Training	Required	Required OK	Required	Required
2. Medical Surveillance (ie Baseline Serology)	Recommended	Required When Appropriate OK	Required	Required

COMMENTS: 1. FIRE EXTINGUISHER IS REQUIRED
2. SPILL KIT & DECONTAMINATION KIT IS RECOMMENDED
3. ACCESS TO AN EMERGENCY PHONE IS REQUIRED.

CDC-NIH Summmary of Containment Levels for Biological Research Prepared by A Jin, Dec 1996

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
HUMAN AND ENVIRONMENTAL PROTECTION COMMITTEES
BOX 0616

HEPC COVER SHEET

PLEASE LIST ALL HEPC REVIEW COMMITTEES TO WHICH THIS PROTOCOL HAS BEEN OR WILL BE SUBMITTED. INCLUDE PERSON LISTED AS PRINCIPAL INVESTIGATOR, APPLICATION TITLE, DATE OF SUBMISSION, AND APPROVAL NUMBER (IF ISSUED). (Whenever possible, the same P.I. and study title should be used on each).

Committee	Principal Investigator	Application Title	Date of Submission	Approval # (if issued)
Committee on Human Research	Joel White	The removal of blood on inanimate dental surfaces by disinfectants	1/19/89	
Committee on Animal Research				
Radiation Safety Committee				
Radioactive Drug Research Committee				
Biosafety Committee	Joel White	The removal of blood on inanimate dental surfaces by disinfectants	1/19/89	

SAN FRANCISCO: SCHOOL OF DENTISTRY
DEPARTMENT OF DENTAL
PUBLIC HEALTH AND HYGIENE

January 19, 1989

TO: Barry Engelstad, MD
Chairman
Committee on Human Research

FROM: Cara M. Miyasaki
Graduate student in Oral Biology

RE: The removal of blood on inanimate dental surfaces by disinfectants

Attached is a request for administrative review of the project named above. I have also attached a short form face sheet with the signature of the Principal Investigator, who is my sponsor for the project.

Thank you for your attention to this matter.

Cara M Miyasaki.

To whom it may concern:

The application for the study: The removal of blood on inanimate dental surfaces by disinfectants will be a study conducted toward completion of a Master's of Science degree in Oral Biology. The attached short form application has partial completion of pertinent information. We wish to claim exempt status for the study.

Thank you,

Cara M. Miyasaki

COMMITTEE ON HUMAN RESEARCH
INITIAL SHORT-FORM APPLICATION

Principal Investigator & Degree (UCSF Faculty) Joel White | University Title Assistant Prof | Dept. Restorative Dentistry
Mailing Address (Campus) D3248, Box 0758 | Phone Number 60918 | Is P.I. sponsor/ advisor only? yes

Co-Investigator Cara M. MIyasaki | Title MS Grad student | Dept. Oral Bio/Stomatology
Mailing Address D1012, Box 0754 | Phone Number 69879, 69889 | Submission Date 1/19/89

Project Title The removal of blood on inanimate dental surfaces by disinfectants

INSTRUCTIONS: Please type. The following should be submitted:
- -1 COPY OF THE HEPC COVER SHEET
- -5 COPIES OF THIS COMPLETED SHORT-FORM
- -5 COPIES OF ALL CONSENT FORMS/INFORMATION SHEETS
- -5 COPIES OF ALL SPECIAL REQUIREMENTS/ATTACHMENTS

NOTE: See PART V of the UCSF GUIDELINES FOR RESEARCH INVOLVING HUMAN SUBJECTS (10/87) for further information and directions on all of the above. Please allow approximately 4-6 weeks for the CHR review process to be completed.

1. STUDY AIM, BACKGROUND AND DESIGN

2. SUBJECT POPULATION: INCLUSION/EXCLUSION CRITERIA, USE OF SPECIAL SUBJECT GROUPS, AND METHODS OF ACCESS

3. PROCEDURES TO BE DONE FOR PURPOSES OF THE STUDY

COMMITTEE ON HUMAN RESEARCH
ADMINISTRATIVE REVIEW FORM (TO CLAIM EXEMPT STATUS)

Investigator Joel White **Address** D3247, Box 0758 **Phone** 60918

Study Title The removal of blood on inanimate dental surfaces by disinfectants **Submission Date** 1/19/89

1. **Give a brief description of this study.** The purpose of this study is to determine if surface disinfectants used in dentistry which are EPA registered and ADA recommended can remove dried blood from various dental surfaces. The experiment will involve the swabbing of three different surfaces with heparinized human blood(blood is obtained from an anonymous donor and screened for infectious agents) which is then left to dry. The effectiveness of the disinfectants to remove dried blood will be detected by applying varius disinfectants to the surfaces. CONT. ON NEXT PAGE

2. **Which exempt category does this research fall within? #___ (From PART VIII-B, UCSF GUIDELINES FOR RESEARCH INVOLVING HUMAN SUBJECTS, October, 1987)**

3. **Will any subjects be contacted for purposes of the study? No X Yes___**
   If so, please describe the contact process.

There will be no subjects involved in this study. The blood is purchased through a local blood bank and the donor is anonymous.

4. **Will subjects be completely anonymous? Yes X No___**
   If not, how will their identities be coded? How long and in what way will records be retained? Who will have access to study data?

There will be no subjects involved in this study.

5. **Will there be any data collection sheets used for study purposes? No X Yes___**
   If so, please submit one copy of each.

CERTIFICATION OF EXEMPT STATUS

On the basis of the information presented here, this research activity qualifies as exempt from review by the Committee on Human Research.

Shan K. Zal 1/24/89

Principal Analyst, Committee on Human Research **Date**
Human & Environmental Protection Committees
Office of Research Affairs, UCSF

DENIAL OF EXEMPT STATUS

On the basis of the information presented here, this study does not qualify for exempt status, and an application should be submitted to the Committee on Human Research for review by the:

___ **SUBCOMMITTEE (for Expedited Review)**
___ **FULL COMMITTEE**

Chairman, Committee on Human Research **Date**

1. Give a brief description of this study CONT.

ıe presence of blood will be detected with a reagent strip which detects the peroxidase-
ike activity of hemoglobin.

Log #_____

UCSF BIOSAFETY COMMITTEE
INFECTIOUS AGENTS APPLICATION - COVER SHEET

This form is for work involving Infectious Agents. (See listing in Appendix A of the Biosafety Guidelines.) Submit ~~10~~ 13 copies of your application to Box 0616

PROJECT TITLE The removal of blood on inanimate dental surface by disinfectants
NAME OF INFECTIOUS AGENT blood
Principal Investigator Joel White
Faculty Title & Dept. Assistant professor Restorative Dentistry
Mailing Address D3248 Box 0758
Phone # 60918
Co/other Investigators (optional) Cara M. Miyasaki
GRANT APPROVAL NUMBER_____ GRANT APPROVAL DATE_____

Please complete the information below in detail. Any omissions may cause delay of the approval.

1. **AGENT DESCRIPTION**
   Attach a brief summary description of the agent, its usual or possible host and vector(s), transmissibility to man and animals, and pathogenicity. What are the implications of possible release of the organism to the environment?

2. **PROTOCOL**
   Attach a description of the protocol, including general experimental design, laboratory safety precautions, containment, decontamination and disposal, procedures for spills, and medical monitoring of personnel.

3. **ANIMALS**
   Will animals be used? Yes___ No X Species_____
   (If animals are used, approval from Committee on Animal Research is also required.)

   Attach a description of the personnel protective measures; animal care and housing precautions; procedures for infecting, handling, and transporting animals, and for containment, decontamination and disposal of infected animals and waste. (A copy of this protocol shall be posted in the animal care facility if it is to be used.) Where will animals be infected and where will they be housed once infected?

4. **LOCATION OF WORK WITH INFECTIOUS AGENT**
   Room(s): D3247
   Classification P2
   (Is room certified? yes Attach a copy of the Inspection & Certification Form)

5. **PERSONNEL INVOLVED**(list names)
   Joel White
   Cara M. Miyasaki

Office Use:

Approval Number_____
Approval Date _____
Expiration Date_____

1/19/89
Date

Joel White
Signature, Principal Investigator

_____
Date

_____
Signature, Chairman, Biosafety Committee

Form revised 10/86

UCSF BIOSAFETY COMMITTEE
INFECTIOUS AGENTS APPLICATION

1. AGENT DESCRIPTION

The agent for the proposed experiment is whole human blood purchased from a private blood bank (Irwin Memorial Blood Bank, San Francisco). The blood is screened prior to distribution for hepatitis B surface antigen, hepatitis B core antibody, the human immunodeficiency virus (HIV) and ALT (SPGT) which screens for non-A and non-B hepatitis. The blood may contain an infectious agent such as HBV and/or HIV if the individual has been recently infected and the antibody titer cannot be detected. Similarly other infectious agents may be present in the blood, however, HIV and HBV are probably the most serious.

The risk of the blood donor specimen containing HIV is approximately 1:50,000. HIV is a fragile organism and cannot live outside the host for very long. Inadvertent contamination of the laboratory environment with the organism may cause infection of susceptible individuals in contact with the organisms.

PROTOCOL

a. .2ml of whole human heparinized blood is pipetted onto two by two inch sterile squares of stainless steel, laminated plastic counter top material (Formica), and naugahyde. The surfaces are sterilized by dry heat or ethylene oxide. A sterile cotton swab is saturated with whole human heparinized blood and used to spread the blood as evenly as possible onto the surface.

b. The surfaces are allowed to air dry for a minimum of 20 minutes and a maximum of 24 hours.

c. After the surfaces are allowed to dry they are a disinfectant is applied by one of three treatments.

    1. One third of the surfaces are treated with a single spray of disinfectant. The surfaces are wiped with a sterile two by two centimeter cotton gauze.

    2. One third of the surfaces are treated with a single spray of disinfectant, wiped with a gauze wipe and sprayed once again. The disinfectant is allowed to dry for ten minutes.

    3. One third of the surfaces are treated with a single spray of disinfectant, wiped with a gauze wipe, sprayed with disinfectant again and wiped with a new gauze wipe.

d. Sterile saline will serve as a negative control. Whole undiluted blood will serve as a positive control.

e. After the surfaces are treated with the disinfectant, the surfaces are tested for the presence of hemoglobin. A reagent strip which detects trace amounts of hemoglobin is moistened with sterile saline. The strip is pressed to the surface of interest three times. After forty seconds the color of the strip is compared to a range of values given by the manufacturer.

f. Latex gloves, gowns, eye protection and masks will be worn during all laboratory procedures. All contaminated disposable items such as gloves, gowns, and contaminated wipes will be placed into a double-bagged biosafety container, sealed and autoclaved for fifteen minutes, 250-275°F.

g. All contaminated surfaces will be either placed into a double-bagged biosafety container, sealed, autoclaved as described above and discarded with appropriate outside markings or placed into a 1:10 solution of sodium hypochlorite overnight, washed with antimicrobial soap and sterilized.

The study investigator will follow university biosafety precautions.

Accidental spills of possibly infectious agents will be properly decontaminated. The area will be flooded with a 1:10 dilution of sodium hypochlorite and left for at least fifteen minutes. The area will be wiped with absorbent paper towels which will be placed in an impervious biosafety container and autoclaved.

In the case of inadvertent splash to mucous membranes or percutaneous exposure, the laboratory technician will be evaluated serologically for evidence of HIV and HBV infection as soon as possible following the exposure. If any acute febrile illness occurs within 12 weeks after exposure, the technician will be advised to report and seek medical evaluation. A lab technician which tests negative for HIV will be retested in six weeks post-exposure, twelve weeks and at six months.

BIOSAFETY COMMITTEE
Division of Human & Environmental
Protection Committees, Box 0616
Office of Research Affairs
University of California, San Francisco

April 15, 1988

To: John S. Greenspan, B.D.S., Ph.D.
Box 0512
x2220

From: the Biosafety Committee

Re: **Status of your Biosafety approval # I-063-01**

Title:
UCSF AIDS TISSUE AND SERUM BANK

In accordance with our responsibility to re-review approved Infectious Agents and Recombinant DNA applications at appropriate intervals, and in order to minimize paperwork and record-keeping, the Biosafety Committee is querying all Principal Investigators with BSC approval dates between 1980-1983 (see attached). Would you please take a few moments to complete this one page questionnaire? If you have any questions, call Kathy Wiemelt at 476-2198.

At your earliest convenience, please return this questionnaire to:
Biosafety Committee
Box 0616

___ This protocol is no longer active and there are no plans to re-activate this study in the future.

___ There is a more current approval that subsumes the above named study. The approval # of the more current study is _____

XX This protocol is currently active.
(attached)
XX An addendum includes a revised list of names for the personnel involved.
___ Any additional findings that would alter the level of containment? If so, then attach an addendum to explain.

Comments_____

5/13/88
John S. Greenspan, B.D.S., Ph.D. date

Re-approved at the June 14, 1988 Biosafety Committee meeting.

Jeffrey Felton
Jeffrey Felton, Chairman, BSC

6/22/88
date

August 3, 1988

DR JOEL WHITE, DDS
RESTORATIVE DENTISTRY
SCHOOL OF DENTISTRY
BOX 0758 D-3247

Re: Laboratory Inspection

As per your request, your laboratory on the third floor of the Dental Building, room 3247 was inspected on the morning of August 3, 1988. The inspection reveal that you will be working with bacteria associated with flora normally isolated from the mouth. The UCSF Biosafety Committee classifies your work to require a P2 containment level.

Upon inspection of your facility, your laboratory conforms with present University and CDC/NIH Guidelines for the P2 Physical Containment Level. All standard microbiological practices shall be strictly enforced. This shall include, but not be limited, to the following:

(1) Eating, drinking, smoking and application of cosmetics in the laboratory are strictly prohibited.
(2) Lab coats are required in the P2 facility and shall not be worn outside the laboratory.
(3) Transport of infectious materials or waste materials for disposal shall be transported in labelled durable leak-proof container(s) with tightly closed lid(s). Autoclave tape shall be used.
(4) The use of an effective chemical disinfectant shall be used at the initiation & termination of each days work.
(5) Access to fire extinguishers, chemical & biological spill kits, and access to telephones are highly recommended in event of emergency situation.

For your records, I have enclosed a copy of (1) the laboratory inspection report, (2) biohazard stickers, (3) a P2 sign, (4) Guidelines for the Preparation of an Emergency Action Plan, and (5) an Index of Audio/Visual Safety Aids.

Laboratory personnel should take care to label and post signs/ stickers correctly. The P2 sign & biohazard stickers shall be displayed in areas where biohazards are present and in a manner that is visible to laboratory personnel, visitors, and students. The purpose of these signs and stickers are to warn all laboratory and non-laboratory personnel to the potential hazard while working in the laboratory. In addition, the needed emergency information will be used to contact individual should the need arise.

Alfred L. Jin
Biohazard Safety Officer

6-0918
6-3028

UCSF LABORATORY INSPECTION REPORT
LABORATORY CONTAINMENT LEVELS FOR BIOLOGICAL RESEARCH

Principal Investigator: DR JOEL WHITE, DDS RESTORATIVE DENTISTRY Bldg/Rm: D-3247

Biohazard: ORAL FLORA, CELL CULTURE Containment Level: P-2

Signed: [signature] Date: CERTIFICATION DATE: AUGUST 3, 1988

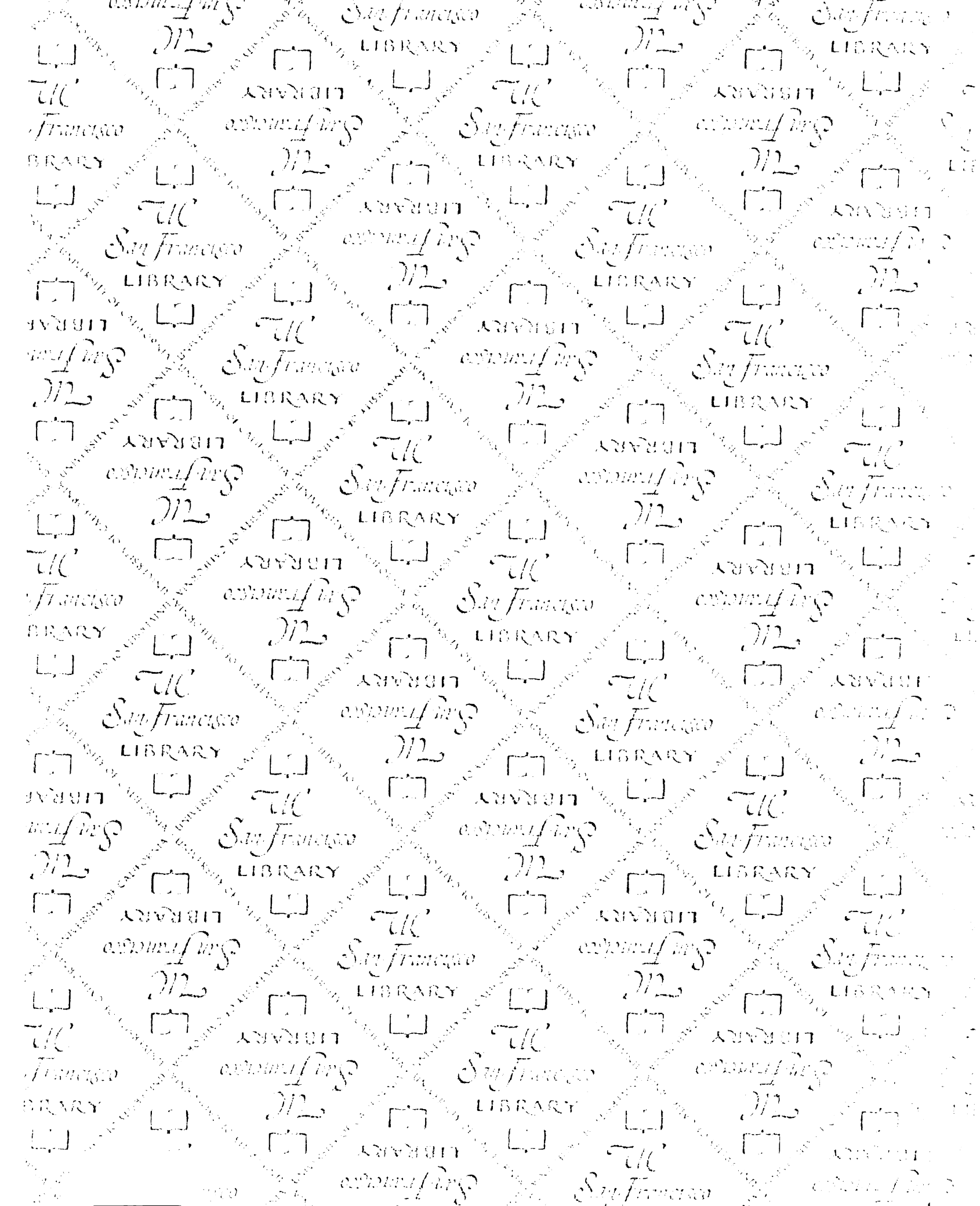
	P1	P2	P3	P4
A. Hazard Levels	Basic/Low Risk	Basic/Low To Moderate	Moderate To High Potential Lethal Consequences	High Risk
B. Standard Microbiological Practices				
1. Public Access While Experiments Are in Progress	Not Recommended	Not Permitted OK	Prohibited/ Controlled	Prohibited/Controlled
2. Daily Decontamination.	Daily & Upon Spills	Daily & Upon Spills OK	Daily, Upon Finished Work And Spills	Daily, Upon Finish Work And Spills
3. Infectious Waste Decontamination.	Before Disposal	Before Disposal OK	Before Disposal	Before Disposal
4. Pipetting.	Mechanical Devices	Mechanical Devices OK	Mechanical Devices	Mechanical Devices
5. Eating, Drinking, Smoking, and Application of Cosmetics.	Not Permitted	Not Permitted OK	Not Permitted	Not Permitted
6. Handwashing Facilities.	Required	Required OK	Required	Foot/Elbow Operations
7. Minimization of Aerosol Production	Recommended	Recommended OK	Recommended	Recommended
8. Laboratory Coats.	Recommended	Required, Not Worn Outside the Laboratory (Is Front Button Coats) OK	Required, Not Worn Outside Laboratory (Is Wrap Around Coat)	Outer Change Room & Shower Facilities Required.
C. Special Practices				
1. Autoclave On-Site Facility	Not Required	Not Required But Available Within Building OK	Required On-Site	Double Door Autoclave Chamber Required On-Site
2. Insect/ Rodent Control Program	Required	Required OK	Required	Required
3. Transport of Infectious Material or Waste Materials For Processing (Is Decontamination) Away From the Lab	Durable Leakproof Container	Durable Leakproof Container OK	Durable Leakproof Container	Non-Breakable Sealed Labeled Primary and Secondary Containers via Disinfection Dunk Tank, Fumigation Chamber/Air Lock

CDC-NIH Summmary of Containment Levels for Biological Research Prepared by A.Jin, Dec.1986.

4. Animals not Involved with Lab Experiment(s)	Not Permitted	Not Permitted OK	Not Permitted	Not Permitted
D. Containment Equipment				
1. Biological Safety Cabinets or other physical containment system	Recommended	Required with Aerosol Generating Equipment, with use of Large Concentrations, Animal Inoculations & Harvesting Infected Tissues from Animals or Eggs. N/A	Required with Aerosol Genera Equipment, Use of Large Concentrations, Inoculation of Animals, Harvesting Infected Tissues from Animals or Eggs and Necropsy of Infected Animals.	Required Use of a Class III Biological Safety Cabinet In Conjunction With a Double HEPA F Exhaust Ventilation Sys Without recirculation or a Special Designed Lab
	Retesting Every 12 months	Retesting Every 12 Months OK	Retesting Every 12 Months	Retesting Every 6 Months
2. Personal Protective Equipment (ie Gloves, mask, surgical respirators & etc.)	Recommended	Required When Handling Infected Animals OK	Required When Handling Infected Animals	Required When Handling Infected Animals
E. Laboratory Facilities				
1. Ventilation	Negative Pressure	Negative Pressure Open Windows Requires Screens OK	Negative Pressure No Recirculation of Air to Other Areas of the Building	Negative Pressure Sensitive Pressure Alarmed Manometers. All Air Exhausted thru HEPA Filt and Discharged Away fr Occupied Buildings and Intake Vents
2. Posted Hazardous Sign	Recommended	Required OK	Required	Required
3. Bench Top Work	Permitted	Permitted OK	Not Permitted	Not Permitted
4. Opened Laboratory Windows	Permitted With Fly Screen	Permitted With Fly Screen OK	Not Permitted	Not Permitted
5. Laboratory Separated From the General Public	No	No OK	Yes	Yes
E. Training				
1. Technical Training	Required	Required OK	Required	Required
2. Medical Surveillance (ie Baseline Serology)	Recommended	Required When Appropriate OK	Required	Required

COMMENTS:
1. FIRE EXTINGUISHER IS REQUIRED
2. SPILL KIT & DECONTAMINATION KIT IS RECOMMENDED
3. ACCESS TO AN EMERGENCY PHONE IS REQUIRED.

CDC-NIH Summmary of Containment Levels for Biological Research Prepared by A.Jin, Dec.1986.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