Risk Assessment for Enteric Pathogens in the Biosafety Level 2 Laboratory

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Abstract

There is some general confusion regarding when to use a biological safety cabinet (BSC) to minimize risk from aerosol formation in Biosafety Level 2 (BSL-2) laboratories that handle enteric pathogens. A risk assessment was conducted to determine the risk involved in performing some standard microbiological manipulations. Although enteric organisms are not known to be infectious via inhalation, it was felt ingestion of droplets deposited around the work area may cause laboratory-acquired infection. Manipulations of Salmonella spp., Shigella spp., Escherichia coli 0157, and other E. coli have resulted in laboratory-acquired infection due to improper laboratory technique. Routine procedures, for example opening screw-capped bottles and wet petri dish covers, improper use of needle and septum, streaking plates, pipetting, slide agglutination, and microscopic preparations, were found to have potential to cause aerosol formation. Proper microbiological technique in combination with primary containment devices (e.g., biological safety cabinets) has been found to reduce the risk of laboratory-acquired infections when working with enteric pathogens. Laboratory workers must ensure that they use any means available to minimize these risks.

Laboratory-acquired Infections

It is obvious that 100% containment cannot be achieved in a microbiology laboratory, even with the best possible technique and equipment. This leads to the potential for a laboratory-acquired infection (LAI). History has shown that such infections occur and that laboratory workers are clearly at higher risk for infection compared with the general population. Over the years our ability to quantify LAIs has been hampered by an indifference to, and perhaps an unwillingness to, report these accidents. Epidemiological methods provide the necessary tools to evaluate the extent and nature of personnel exposures in the absence of precise data on LAIs. Acquisition of this invaluable information may prevent potential exposures by the implementation of appropriate work practices and safety equipment, improvement of facilities, and the rigorous training of technical and support personnel.

Estimates of the extent of LAIs are imprecise. Information available from publications, questionnaires, and personal communication usually summarize acute symptomatic infections with minimal data on seroconversion or nonsymptomatic response to laboratory-associated microorganisms. Rarely was the host immune competence considered. Assessing the true incidence of LAIs is impossible without any centralized reporting of infections and routine assessment of worker exposure (Fleming et al., 1995).

Today there is a much more comprehensive understanding about the infectious nature of and modes of transmission of microorganisms, yet a substantial number of accidental LAIs are still encountered. Infection with BSL-2 agents have involved considerable medical expense and time-loss (3.17 days per infection; Sullivan et al., 1978).
Risk Assessment

Risk assessment is an integral procedure in the control of LAIs. The interrelated notions of “hazard” and “risk” are part of this process. The “hazard” presented by a substance is the potential to cause harm in some way (e.g., to cause an infection). The “risk” is the likelihood that it will cause harm in the circumstances under consideration (e.g., give rise to an infection in a microbiological laboratory worker). In terms of harm presented, a risk can be perceived as “vanishingly small” and acceptable or as being so severe as to make it totally unacceptable. Deciding what constitutes an acceptable risk is a management task; a risk that is acceptable in one set of circumstances may be unacceptable in another (Collins & Kennedy, 1999).

The development of programs to minimize risks associated with the handling and disposal of infectious agents is dependent on an understanding of the pathogenicity of the agent, the susceptibility of the host, and, most importantly, the method of transmission of the infectious agent.

Most risk from biological hazards can be reduced through engineering controls, personal protection, the use of appropriate microbiological techniques and procedures, and decontamination. Engineering controls in the laboratory include control of the laboratory ventilation (e.g., negative pressure laboratories), biological safety cabinets, sealed centrifuge rotors and buckets, and other devices designed to minimize exposure to infection. Personal protective devices available for the laboratory include laboratory coats and surgical gowns, gloves, safety glasses and visors, and occupational health services including vaccination and immunization.

Appropriate microbiological techniques include standard operating procedures that incorporate “universal precautions” (e.g., avoidance of “sharps”; rules prohibiting eating, drinking, smoking and application of cosmetics in the laboratory; and requiring regular hand-washing) designed to minimize exposure to infection. Each laboratory should use an appropriate disinfectant based on its efficacy against the organisms encountered therein.

Routes of Infection

In order to perform a risk assessment in the laboratory it is extremely important to consider that exposure to infectious agents can occur by several routes. These include:

A. Inhalation: Activities that generate aerosols
   1. Manipulating needles and syringes (Figure 1)
      A. Expelling air from tubes or bottles
      B. Withdrawing needles from stoppers
      C. Separating needles from syringes

   Figure 1
   Aerosol formation (withdrawing needle from stopper).

   Courtesy of Gillian Norton, CRSP, RBP, Biosafety Officer, University of Western Ontario

   2. Manipulating inoculation needles or loops
      A. Flaming loops
      B. Cooling loops on inoculated portion of culture media
      C. Subculturing and streaking culture media

   3. Manipulating pipettes
      A. Mixing microbial suspensions
      B. Spilling microbial suspensions on hard surfaces

   4. Manipulating specimens and cultures
      A. Centrifugation
      B. Mixing, blending, grinding, shaking, sonication, and vortexing of specimens or cultures
      C. Pouring or decanting fluids
      D. Removing caps or swabs from culture containers
      E. Spilling infectious materials
      F. Filtering specimens under vacuum
B. Ingestion: Activities related to oral transmission
   1. Pipetting by mouth
   2. Splashing contaminated material into the mouth
   3. Placing contaminated material or fingers in the mouth
   4. Eating, drinking, using lipstick, and smoking in the workplace

C. Inoculation: Activities related to direct intravenous and subcutaneous transmission
   1. Manipulating needles and syringes
   2. Handling broken glass, scalpels, and other sharp objects

D. Inoculation: Activities related to contaminated skin and mucous membranes
   1. Splashing or spilling material into eyes, mouth, and nose onto skin
   2. Exposing nonintact skin to contaminated material
   3. Working on contaminated surfaces
   4. Handling contaminated equipment
   5. Inappropriate handling of loops, inoculating needles, or swabs containing specimen or culture material (Isenberg, 1998)

Pathogens that are not normally handled may be introduced into reference laboratories by the receipt of unknown specimens. These pathogens could be infectious through almost all routes of infection.

Agent-specific Factors

Agent-specific factors also must be considered. These include increased virulence, antimicrobial drug resistance, and working with high concentrations of microorganisms. Working with microorganisms at high concentrations increases the risk of laboratory-acquired infection, thus necessitating more stringent precautions (Collins & Kennedy, 1999).

*Escherichia coli* provides a good example of the problem of increased virulence. In the United Kingdom, this organism is assigned to Risk Group 2, with the exception of nonpathogenic strains, which would be assigned to Hazard Group 1 (ACDP, 1995a). However, verotoxin-producing *E. coli* 0157:H7 may cause severe human disease, may have an infective dose as low as 10 organisms, and may cause laboratory-acquired infection. Accordingly it has recently been promoted to Group 3 (HSE, 1998a).

Multiple-drug resistant microorganisms present another challenge for the laboratory worker. A laboratory-acquired infection with an organism that has multiple-drug resistance is more difficult to treat and therefore results in more serious consequences for the affected person. An example of an enteric microorganism that falls into this category is methicillin-resistant *Staphylococcus aureus*.

Host-specific Factors

Of equal importance are host-specific risk factors. These can be divided into two classes—intrinsic and extrinsic factors. Intrinsic factors include immunodeficiency, immunosuppression, pregnancy, and old age. Extrinsic factors include habits such as nail biting and pen chewing that increase the risk of infection through the mouth, and wearing contact lens that increases the risk of localized eye infection and systemic infection via the eye. *E. coli* is also recognized as a potential pathogen of the eye (Ready, 1998).

The Enterics Laboratory

Scientific literature (e.g., Sewell, 1995) documents that enteric microorganisms are not infectious by inhalation but become hazardous when they are ingested. Laboratory studies of potential sources of infection have focussed on hazards associated with routine microbiological techniques. As previously discussed, aerosols can be produced by almost all routine bacteriological and virologic procedures and present two means of potential worker exposure: through minute respirable airborne particles and by the disposition of larger heavy droplets onto surfaces, equipment, and personnel (Fleming et al., 1995) (Figures 2 and 3). It is these larger, heavier aerosols that must be considered in a risk assessment of routine procedures in the enterics laboratory.

Fingers are frequently contaminated during laboratory manipulations, even by the most careful workers. Benches and equipment may be contaminated as mentioned above, by the deposition of aerosols or as a result of unrecognized spillage of cultures. The organisms are picked up on the fingers or articles such as pens, pen-
cils, and paper and transferred, directly or indirectly, to the mouth (Collins & Kennedy, 1999).

There have been numerous studies of aerosol production during laboratory operations. These have included centrifugation (Reitman & Wedum, 1956; Rutter & Evans, 1972; Whitwell et al., 1957), use of AutoAnalyzer equipment (Rutter & Evans, 1972), opening screw-capped bottles (Tomlinson, 1957), and various bacterial culture techniques (Andersen et al., 1952; Johansson & Ferris, 1946; Reitman et al., 1954; Reitman & Wedum, 1956). The "tracer" organism used in these studies has usually been a distinctive bacterium, occasionally a chemical substance. Both aerosol production and surface contamination were observed. Although some of these studies were conducted almost 50 years ago, the message is still current because traditional microbiology techniques have not changed significantly.

**Figure 2**
Formation of minute respirable airborne particles (pipette).

**Figure 3**
Deposition of heavier droplets on a hard surface.

**Table 1**
Relative Contamination Hazards of Laboratory Operations

<table>
<thead>
<tr>
<th>TYPE OF OPERATION</th>
<th>AEROSOL*</th>
<th>SURFACE CONTAMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation</td>
<td>Nil†</td>
<td>Yes</td>
</tr>
<tr>
<td>Vortex mixing</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>Opening containers</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>Pasteur pipette</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>Controlled pouring</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Oxford pipette</td>
<td>25</td>
<td>Yes</td>
</tr>
<tr>
<td>Spilling on floor</td>
<td>104</td>
<td>Yes</td>
</tr>
<tr>
<td>Shaking (paint can; vacutainer tube)</td>
<td>&gt;1,000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Average number of particles <5 μm diameter observed per operation (plaques counted on stages 4, 5 and 6 plus 50% of plaques on stage 3 of Andersen Sampler).

† Unless tube breaks in centrifuge (Reitman, et. al., 1956) or liquid passes through narrow opening (Rutter, et. al., 1972).
Standard Techniques

Stern et al. (1974) found considerable bench-top and instrument-surface contamination associated with all procedures (Table 1).

A more in-depth discussion of some standard techniques that may produce aerosols in the enterics laboratory follows.

1. **Streaking Plates**

   The use of a microbiologist’s loop is a common source of aerosol generation and subsequent contamination of laboratory surfaces. Procedures that generate aerosols and contaminate surfaces include the spontaneous discharge of liquid from a loop, the streaking of media (particularly media with a rough surface), “cooling” a loop on the inoculated portion of culture media, and heating a loop in an open flame. Sewell (1995) mentions that the use of a BSC can decrease this risk when working with hazardous microorganisms.

2. **Biochemical Identification**

   A number of procedures are routinely used for the biochemical identification of enteric microorganisms. The following is a short, but not exhaustive, discussion of procedures that have been found to cause aerosols in the laboratory and subsequent contamination of the laboratory environment and workers.

   i. **Pipetting**: Pipetting is a time-honored laboratory technique that is a potential hazard (Collins, 1993; Hanel et al., 1986). The risks associated with pipetting include ingestion via mouth pipetting, inhalation via aerosols produced by mixing a microbial suspension or spilling drops on hard surfaces, contamination of bench tops and fingers, and injuries from broken glass pipettes.

   In an experiment reported by Stern et al., (1974), a pasteur pipette was used to drop phage-containing solution (a 1:1 mixture of serum and broth) onto a glass microscope slide. When dropped 1.5 cm, no splattering was observed, but when the height was increased to 7 cm, 10 plaques were formed (sedimentation plate directly below slide).

   ii. **Centrifugation**: Centrifuge accidents cause relatively few laboratory-associated infections, but a single accident often exposes a large number of individuals. Unrecognized releases of aerosols during centrifugation may be responsible for laboratory-acquired infections without an identifiable source. The centrifuge safety cup must be opened in a BSC after centrifugation (Collins, 1993; Kruse et al., 1991).

   iii. **Other Procedures**: Other hazardous procedures are also routinely performed in the microbiology laboratory. For instance, if a film of liquid exists between two surfaces that are separated (e.g., when removing a petri dish cover or test tube cap), an aerosol may result. Liquids hitting a hard surface (breakage or spillage) create large aerosols and contamination of the environment. Blood and clinical material spread over a microscopic slide may be a potential biohazard. Most of these hazards are minimized by enclosing the opening of the container with a disinfectant-soaked pledget or placing the containers in a BSC before opening them (Sewell, 1995).

3. **Slide Agglutination and Microscopic Preparations**

   When a loopful of a liquid culture is spread on a slide or a suspension is made on a slide from a solid culture, small droplets may be broadcast, particularly if the loop is wielded energetically. When the loop is withdrawn from the drop, more small droplets may be scattered and aerosols may be formed. This was demonstrated by Hirschbruck and Forthman (Fricke, 1919) who made smears on cover glasses placed on the surface of culture media that were then incubated. Price (1976) sampled the bench on which slide agglutinations with *Salmonellae* and *shigellae* were performed. Swabs and contact plates were used to sample the bench after 18-200 slide agglutinations were completed. *Salmonellae* were recovered from as many as 28 of 30 samplings and *shigellae* from 15 of 22. Aerosol formation during slide agglutination tests was also studied by Reitman and Wedum (1956) who recovered an average of 0.3 colonies per operation from the air during 60 slide agglutinations.

   Dadswell (1983) reported on two cases of laboratory-acquired infection pertaining to slide agglutination:

   1. A wooden applicator broke while colonies of *Shigella sonnei* were being transferred to a tube of saline for agglutination tests. Despite discarding her protective clothing, washing her hands thoroughly, and cleaning the bench, the person concerned developed diarrhea
about 48 hours later: S. sonnei was isolated from a sample of stool.

2. Another infection with S. sonnei was apparently the result of slide agglutination tests being done by an inexperienced worker without adequate supervision. In this case the individual also became infected with Salmonella paratyphi B, with which she had also been working.

The dangers of slide agglutination have also been noted in a publication by Collins, Hartley, and Pilsworth (1974). Price (1976) conducted further investigations shown in Tables 2 and 3.

Price (1976) states that it is probably advisable not to perform more than six agglutinations on one slide, as the chance of environmental contamination will likely increase in proportion to the number of agglutinations done.

Microscopical preparations with particularly hazardous organisms should be done in a BSC. Once these preparations have been made, the slides may be brought out of the cabinet to dry, fix, and stain. The hazard from droplets and aerosols is then replaced by that of contamination of the fingers (Collins & Kennedy, 1999).

### Table 2

**Salmonella Isolations from Personal and Laboratory Environment (slide agglutination test)**

<table>
<thead>
<tr>
<th>SURFACE SAMPLED</th>
<th>TOTAL SAMPLES</th>
<th>POSITIVE SAMPLES</th>
<th>SEROTYPES ISOLATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectacles</td>
<td>24</td>
<td>0</td>
<td>S. agona</td>
</tr>
<tr>
<td>Fingers</td>
<td>6</td>
<td>2</td>
<td>S. anatum</td>
</tr>
<tr>
<td>Tile swabs</td>
<td>30</td>
<td>28</td>
<td>S. breedeney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. cubana</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. eimsbuettel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. hadar</td>
</tr>
<tr>
<td>Tile contact plate</td>
<td>27</td>
<td>17</td>
<td>S. heidelberg</td>
</tr>
<tr>
<td>Bench surrounding tile</td>
<td>28</td>
<td>6</td>
<td>S. liechfield</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. typhimurium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. tennesse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. senftenberg</td>
</tr>
</tbody>
</table>

### Table 3

**Shigella sonnei Isolations from Personal and Laboratory Environment (slide agglutination test)**

<table>
<thead>
<tr>
<th>SURFACE SAMPLED</th>
<th>TOTAL SAMPLES</th>
<th>POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fingers</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Tile swabs</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Tile contact plate</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Tile surround</td>
<td>22</td>
<td>1</td>
</tr>
</tbody>
</table>
Other LAIs in the Enterics Laboratory

Following is a brief, but not exhaustive, synopsis of LAIs that were reported for some enteric organisms.

A. Salmonella

Pike (1976, 1978) extensively reviewed the incidence of laboratory-acquired typhoid fever in a survey of all types of laboratory-acquired infections occurring worldwide from 1930 to 1974. Salmonella typhi accounted for 258 cases (6.3%) out of a total of 4,079 cases of laboratory-acquired infections involving different bacteria, viruses, fungi, and parasites (ranked fourth in the list of 10 most common agents next to Brucella spp. and Coxiella burnetii). In Pike’s survey typhoid fever accounted for more fatalities than any other laboratory-acquired infection (80 deaths, or a 7.8% mortality rate). This indicates that S. typhi is a potentially fatal infectious hazard to laboratory personnel.

Collins (1983) summarized the modes of transmission of S. typhi in the microbiological laboratory. These include mouth pipetting and hand-to-mouth transfer from contaminated surfaces. He found that the latter mode of transmission occurs most frequently by (1) subculturing from enrichment media, (2) plating, (3) slide agglutinations, and (4) pipetting.

Other examples of typhoid fever acquired from such manipulations in the United States were reported in a number of publications (Blaser & Feldman, 1980; Blaser & Lofgren, 1981; Holmes et al., 1980). Blaser et al. (1980) have also pointed to the laboratory as a reservoir of infection by S. typhi. During the period from 1977 to 1979, laboratory-acquired cases represented 11.2% of the sporadic typhoid cases reported to the United States.

Many laboratories maintain their own stock cultures, which are periodically subcultured. Collins and Kennedy (1999) found that infections arising from this procedure seem to be confined to salmonellae, especially S. typhi. Olson et al. (1961) found that a strain isolated 41 years earlier was still able to infect a worker. The Centers for Disease Control and Prevention (1979a) reported 11 cases arising from stock cultures. Blaser and Lofgren (1981) told of a laboratory worker who handled a stock strain and transmitted it to two members of his family without becoming infected himself.

Occupational exposure was the probable cause of three infections by Salmonella typhimurium and one by S. typhi, all affecting medical laboratory scientific officers in microbiology (Grist & Emelie, 1989). Ashdown and Cassidy (1991) describe a case of laboratory-acquired typhoid fever in which the mode of transmission of the S. typhi was presumed to be a laboratory infection from an unknown source. Although there was no obvious breakdown in safe laboratory techniques, the infecting dose of S. typhi is known to be small and the dangers of handling specimens that may contain this bacterium are emphasized.

In another case, a laboratory worker, known previously to routinely practice careful microbiological technique, received S. typhi as an unknown in a simulated stool specimen. The specimen was cultured onto solid media and into liquid enrichment broth. The broth was subcultured and the organism was identified biochemically by conventional tests with confirmation by serological agglutination reactions. In addition to these potential modes of transmission, Hoerl et al. (1988) believe that other factors may have been important in acquisition of S. typhi by this student. These include:

1. the absence of a biological safety cabinet;
2. the lack of foot pedals on sinks for hand-washing in the student laboratory; and
3. student access to the instructor’s laboratory where the unknown samples were prepared.

Although 105 S. typhi is the reported infective oral inoculum (Hornick, et al., 1970), it is known that larger numbers of bacteria may be easily found in colonies from bacterial culture plates and in drops of broth media.

Carelessness and poor techniques are the major cause of laboratory accidents (CDC, 1979). Because of the risk of infection not only to themselves but also to others, microbiologists must be especially scrupulous about adherence to recognized standards of safety (U.S. Department of Health, Education, and Welfare, 1979).

The World Health Organization (Blaser & Lofgren, 1981; WHO, 1980) has classified S. typhi as a Risk Group III (high individual risk and low community risk) organism and stipulates that the organism should be handled only in specialized laboratories with restricted access.
B. Shigella

The literature abounds with evidence of laboratory-associated infections with shigellae. Jacobsen et al. (1985) reported a case of Flexner dysentery acquired from a quality control specimen.

In a survey of 166 British clinical laboratories (Crist & Emslie, 1991), Shigella infections were the most common laboratory-acquired infections; however, the mechanism(s) of transmission was not elucidated. Interestingly, three of the Shigella infections reported in this survey originated from quality control specimens. The same authors have also provided data suggesting that Shigella flexneri is more easily transmitted in the laboratory setting than is Shigella sonnei.

Ghosh (1982) reported that S. flexneri has the smallest infecting dose of enteropathogenic bacteria—namely, about 200 viable cells for healthy volunteers—so that infections in laboratory workers are not uncommon (Hornick, 1977). In general, laboratory-acquired infections of all kinds have occurred most often in trained bench workers in diagnostic services, and spills and sprays have equaled hypodermic needles as the commonest mode (Pike, 1979) of transmission.

Crockett et al. (1996) found that the median infectious dose (ID50) for Shigella species that were examined appeared to be around 1,000 organisms, and the dose-response curve was consistent with the beta-Poisson model that was developed. There appeared to have been outbreaks in which the average dose ingested was in the order of 10 organisms.

Collins and Kennedy (1999) describe a case of laboratory-acquired shigellosis in which the only definite risk factor that could be identified was that the individual concerned was a nail-biter. Frequent involuntary contacts between the potentially contaminated fingertips and the lips and the teeth occur when biting one's fingernails. It is easy to see how minute traces of material that could contain a minimum infective dose of Shigella spp. could be transmitted in this way. Finger-licking while page-turning as well as pen-chewing are other habits that may cause laboratory workers to inadvertently infect themselves while in the laboratory.

Mermel et al. (1977) describe an outbreak of S. sonnei infection among medical technologists in a hospital microbiology laboratory. This is one of the largest reported outbreaks of shigellosis in a hospital laboratory. A number of events may have culminated in this outbreak. Namely, a quality control strain of Shigella was subjected to prolonged handling by a student over several days when the hand-washing sink in the laboratory had been changed from foot pedal to faucet handle operation. Transmission appears to have resulted from contamination of the faucet handles of the hand-washing sink. The student working with the unknown isolate admitted that heavy glove contamination had occurred when he placed his gloved finger in a titre well containing a high concentration of S. sonnei during the typing process. When the student was further questioned, it became evident that other breaches in technique had occurred. This case serves to further emphasize that written protocols and appropriate training in sterile technique may not ensure good practice. Despite the rigorous training of students and new personnel, close supervision of these individuals is of paramount importance. Also, with experienced employees "protocol drift" is not uncommon in laboratories when supervision is poor and standard operating procedures are not under continuous review (Collins & Kennedy, 1999).

C. E. coli 0157

Although the absolute numbers of infections are small in comparison with other enteric pathogens such as Salmonella spp. or Campylobacter spp. (Coia, 1998), it is well recognized that E. coli 0157 has the propensity to cause severe, life-threatening human infections.

The well-documented occurrence of waterborne transmission associated with contaminated drinking supplies (Dev et al., 1991; Swerdlow et al., 1992) and recreational use of water (Brewster et al., 1994; Keene et al., 1994), the high rate of secondary infection in outbreaks (Advisory Committee on Microbiological Safety of Food, 1995; Coia, 1998), and the occurrence in laboratory-acquired infection, all suggest that the infectious dose for this organism is small (Todd et al., 1988). Quantitative culture of foodstuffs epidemiologically implicated in episodes of infection has demonstrated levels of contamination as low as two organisms per 25 g food (Willshaw et al., 1994). Also, recent in vitro studies have demonstrated that some strains remain viable on stainless steel surfaces in excess of 35 days (Maule, 1997) as well as on the surface of hard
currency (Jiang & Doyle, 1999).

In 1993, Burnens et al. presented a case where a laboratory worker acquired an infection with *E. coli* 0157:H7, even though there were no obvious technical procedural errors and the worker was performing her work inside a biological safety cabinet. They also concluded that the infectious dose for *E. coli* 0157:H7 must be low. Further evidence of this is supplied by the fact that *E. coli* 0157:H7 may be transmitted directly from person-to-person (Carter et al., 1987).

A case of *E. coli* 0157 infection (confirmed by stool culture and a high titre of serum antibodies to the lipopolysaccharide of *E. coli* 0157; Chart et al., 1989) occurred in a previously healthy laboratory technician who subsequently developed severe renal failure requiring hemodialysis. The technician had handled strains of *E. coli* 0157 daily before the onset of diarrhea. Work with the bacteria had included subculturing strains, doing agglutination reactions, and opening broth cultures that had been steamed. There was no history of a laboratory accident or known lapse in good technique (Booth & Rowe, 1993).

In 1998, a laboratory worker was self-infected while doing serology on *E. coli* 0157:H7 on the laboratory bench (personal communication, 1998). To prevent future incidents of this nature, the biosafety officer recommended that serotyping be performed in a biological safety cabinet, to make sure bench tops were properly disinfected, and to review hand-washing procedures.

Rao et al. (1996) discuss a serious laboratory-acquired infection with *E. coli* 0157:H7 in a laboratory worker. They feel that in light of this and two other previously published reports, the hazard category of this organism should be reexamined (Booth & Rowe, 1993; Burnens et al., 1993).

Owing to the known hazards and to minimize the risk of future occupational exposure of laboratory staff, the Advisory Committee on Dangerous Pathogens in the United Kingdom concluded that verotoxigenic *E. coli*, including *E. coli* 0157, should be reclassified as Hazard Group 3 organisms (but with a facility for derogation from full containment level 3), rather than Hazard Group 2 (which is the case for other *E. coli*). The proposal to the European Commission was formally adopted on October 7, 1997 (97/59/EC) for implementation by member states by May 1, 1998. It is likely that the final advice will recommend that verotoxigenic *E. coli* should be subjected to derogation from the full Hazard Group 3 precautions in a similar manner to the existing guidance for *Salmonella typhi* and *Shigella dysenteriae* Type 1 (Coia, 1998).

**D. Other *E. coli***

Parry et al. (1981) report what they believe to be the first proved example of a urinary tract infection due to a strain of *E. coli* acquired in the laboratory. It was assumed that the patient had unwittingly ingested *E. coli* SP88 in the laboratory and that her intestinal tract had thus become colonized, and that her urinary tract infection had come about by the accepted route (Sussman & Asscher, 1979). The laboratory strain with which the technician was working was identical to the strain isolated from her urine, but *E. coli* with the required characteristics could not be detected in her fecal flora. The researchers could not exclude that she had transferred the infecting strain to the introitus from her fingers.

**Summary**

The Laboratory Safety Guidelines (Kennedy, 1996) for Canada state that Class I or II biosafety cabinets are required for all manipulations of agents that may create an aerosol. The Centers for Disease Control/National Institutes of Health (Richmond & McKinney, 1999) also concur. They maintain that even though organisms routinely manipulated at Biosafety Level 2 are not known to be transmissible by the aerosol route, procedures with high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment such as a biological safety cabinet or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Although the recommendations proposed by these groups are not mandated by law, they do represent a consensus of opinions by peers and therefore define a "standard of practice" that laboratories should follow. Current information on the effectiveness of universal precautions for reducing risks suggests that adherence to the guidelines promulgated by various regulatory agencies decreases the risk of occupational exposure to
infectious agents and therefore contributes to a safer work environment (Gilchrist et al., 1992; Sewell, 1995; U.S. Department of Health and Human Services, 1993; Wong et al., 1991).

Facility management is responsible for the development and institution of safety procedures and employee training programs that minimize the employee risk from a laboratory-associated infection on the basis of present or anticipated infectious hazards. The strategy for minimizing the occupational exposure of laboratory workers, other facility employees, and the surrounding environment to infectious agents is based on the concept of microorganism containment, which includes physical factors (e.g., facility design and safety equipment), standard microbiological practices, and administrative controls (Fleming et al., 1995; Gershon & Salkin, 1992; Gilchrist et al., 1992; National Committee for Clinical Laboratory Standards, 1994; U.S. Department of Health and Human Services, 1993).

Primary containment provides physical separation of the infectious agents from the laboratory worker. Primary barriers include strict adherence to microbiological practices and techniques and use of safety equipment such as biological safety cabinets, safety centrifuge containers, and personal protective equipment (e.g., gloves, masks, face shields and glasses, coats, and gowns). Secondary containment refers to the facility design and acts as a secondary barrier to protect all workers within the facility and to protect the outside environment (Sewell, 1995).

It has been shown in this risk assessment that most routine procedures that are carried out in the Biosafety Level 2 enterics laboratory can be expected to cause aerosols and therefore contaminate the laboratory and the worker. Since many of the organisms dealt with in the enterics laboratory are serious human pathogens that are infectious by ingestion at very low doses, it would seem prudent to heed the opinion of experts in the world of biosafety and move all aerosol-producing procedures back into the biological safety cabinet before being faced with a laboratory-acquired infection.

Public sensitivity regarding the risk of infection has increased in today's world, although it must also be noted that there are differences in opinion between the laboratory and the public about the acceptability of risk. Therefore, the onus is on laboratory management to allay fears of infection by demonstrating that every possible containment measure is reliably in place and to provide all the supporting documentary advice that is necessary.

References


