It is vitally important that once the gloves are on, operators become aware of everything that comes into contact with their hands. Touching the face, head, and hair will contaminate both sterile and nonsterile (disinfected) gloves with a large bioburden. If this occurs, a new set of gloves should immediately be put in place. Management must be very aware of this habit and work hard to train the staff to be selfaware and break the habit in order to maintain a high level of aseptic operations. It cannot be said enough: No touching!

Similarly, once the gloves have been donned, staff must be aware of what those gloves come into contact with. Not all reagents, tools, equipment, or even surfaces will be sterile. They may all carry different bioburden risks. There should be general guidance in any facility and specific protocols on what staff can and cannot touch once the gloves used for the procedure are donned. This may require gloves to be changed, or at least disinfected with 70% alcohol again.

Of course, bare hands should never come into contact with surfaces in the laboratory, outside of specially designated areas, if at all. Some laboratories do maintain an administrative or paperwork area where no biologicals will be kept or come near. It is usually recommended that, at the very least, nonsterile gloves should be used when hands are in contact with other areas of the room. For example, in the case of flow cytometry, gloves should be used when manipulating the reagents and cell solution, as well as when using the keyboard.

No matter how efficient a cleaning and disinfecting program is, it may miss an area, and that poses risk for the staff. In addition, touching bare skin to any laboratory surface will contaminate the surface with the resident microbiome.

## **Use of Common Laboratory Tools**

In addition to being aware of what surfaces an operator's hand and gloves touch, it is important to be aware of proper tool use. If a sterile item touches any surface, it should be considered potentially contaminated, and replaced with a new sterile item. For example, someone might use a 10-mL serologic pipette to perform a media fill, but accidentally touches the side wall of the BSC with it. That pipette is now considered nonsterile and must be replaced. The risk of contaminating an entire process or production run is too great compared to the price of a single tip, pipette, or other tool. Even if the area has been disinfected, a risk remains that something may have been missed during cleaning, microbial risk may have been introduced during setup, or contamination may have occurred during the procedure. The same rule applies to a reusable stainless steel cannula or other similar item; swapping for a new, sterile item is the safest option. Ensuring that extra disposables are easily available is a good option to minimize risk when minor accidents happen.

Extra care should be taken during handling of tubing, liquid or biological reagent bags, or anything with small caps or access ports. By their nature, these items require workers to place their hands on or close to areas that will, in turn, be in close proximity to, or in contact with, biologicals or sterile fluids. Care must be taken to ensure no inappropriate contact occurs when handling the ports and spikes for these items.

## Tubing

Some laboratories use sterile forceps or similar tools to manipulate the openings at the end of tubing. Holding the tubing aloft inside the BSC can help maintain a "no-touch" workflow. However, if either end of the tubing must be placed down, the access port or spike needs to remain elevated so as not to touch any other surface in the BSC. (Fig 4-2). The same is true for the access ports on blood bags or other reagent bags, as the ports will be the most likely route of contamination. Handle with care!

## Bottles and Conical/Test Tubes

Bottles and tubes (test tubes, conical tubes, etc) should be opened only as they are being used, and closed or capped directly after use has ended. Keeping a cover on the bottle or tube prevents the risk of contamination from someone passing something over the bottle, disruption of airflow, generation of aerosols, and nearly any event that might "drop" potential contamination into the sterile bottle or reagent inside.

If it is necessary for a bottle or tube to remain open due to workflow considerations, then the cap should be held in hand if at all possible,



and replaced at the operator's earliest convenience. Holding the cap poses the lowest risk of accidental contamination.

Sometimes workflow will demand the cap or lid be placed down, often due to repeat access or the operation requires both hands. In that case, the risk assessment of "cap up" or "cap down" must be weighed (Table 4-1).

For "cap up" the opening (inside) of the cap is placed facing up, so it is exposed to the sterile air of the BSC. The benefit is that the cap will not drip or have any inherent aerosols drop onto the BSC. Conversely, there is increased risk of something accidentally being passed over the face-up cap. This risk can be mitigated by placing the cap well out of the workflow area.

For "cap down" the opening of the cap is placed facing down onto the BSC workspace. The benefit here is that the risk of contaminating the inside of the cap due to aerosols or spills is nearly completely mitigated. However, in that position the inside of the cap may transfer any residue of liquids or reagent onto the BSC surface and contaminate it. Also, if the BSC surface has any contamination, from missed/ improper disinfection, the cap may pick up that contamination and transfer it back to the bottle or tube.