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Sterilizing practices from the Guideline for Disinfection and Sterilization in Healthcare Facilities (2008). The delivery of sterile products for use in patient care depends not only on the effectiveness of the sterilization process but also on the unit design, decontamination, disassembling and packaging of the device, loading the sterilizer, monitoring, sterilant quality and quantity, and the appropriateness of the cycle for the load contents, and other aspects of device reprocessing. Healthcare personnel should perform most cleaning, disinfecting, and sterilizing of patient-care supplies in a central processing department in order to more easily control quality. The aim of central processing is the orderly processing of medical and surgical instruments to protect patients from infections while minimizing risks to staff and preserving the value of the items being reprocessed⁹⁵⁷. Healthcare facilities should promote the same level of efficiency and safety in the preparation of supplies in other areas (e.g., operating room, respiratory therapy) as is practiced in central processing. Ensuring consistency of sterilization practices requires a comprehensive program that ensures operator competence and proper methods of cleaning and wrapping instruments, loading the sterilizer, operating the sterilizer, and monitoring of the entire process. Furthermore, care must be consistent from an infection prevention standpoint in all patient-care settings, such as hospital and outpatient facilities. A sterilization process should be verified before it is put into use in healthcare settings. All steam, ETO, and other low-temperature sterilizers are tested with biological and chemical indicators upon installation, when the sterilizer is relocated, redesigned, after major repair and after a sterilization failure has occurred to ensure they are functioning prior to placing them into routine use. Three consecutive empty steam cycles are run with a biological and chemical indicator in an appropriate test package or tray. Each type of steam cycle used for sterilization (e.g.,

vacuum-assisted, gravity) is tested separately. In a prevacuum steam sterilizer three consecutive empty cycles are also run with a Bowie-Dick test. The sterilizer is not put back into use until all biological indicators are negative and chemical indicators show a correct end-point response^{811-814, 819, 958}. Biological and chemical indicator testing is also done for ongoing quality assurance testing of representative samples of actual products being sterilized and product testing when major changes are made in packaging, wraps, or load configuration. Biological and chemical indicators are placed in products, which are processed in a full load. When three consecutive cycles show negative biological indicators and chemical indicators with a correct end point response, you can put the change made into routine use^{811-814, 958}. Items processed during the three evaluation cycles should be quarantined until the test results are negative. The central processing area(s) ideally should be divided into at least three areas: decontamination, packaging, and sterilization and storage. Physical barriers should separate the decontamination area from the other sections to contain contamination on used items. In the decontamination area reusable contaminated supplies (and possibly disposable items that are reused) are received, sorted, and decontaminated. The recommended airflow pattern should contain contaminants within the decontamination area and minimize the flow of contaminants to the clean areas. The American Institute of Architects ⁹⁵⁹recommends negative pressure and no fewer than six air exchanges per hour in the decontamination area (AAMI recommends 10 air changes per hour) and 10 air changes per hour with positive pressure in the sterilizer equipment room. The packaging area is for inspecting, assembling, and packaging clean, but not sterile, material. The sterile storage area should be a limited access area with a controlled temperature (may be as high as 75°F) and relative humidity (30-60% in all works areas except sterile storage, where the relative humidity should not exceed 70%).⁸¹⁹ The floors and walls should be constructed of materials capable of withstanding chemical agents used for cleaning or disinfecting. Ceilings and wall

surfaces should be constructed of non-shedding materials. Physical arrangements of processing areas are presented schematically in four references^{811, 819, 920, 957}. As repeatedly mentioned, items must be cleaned using water with detergents or enzymatic cleaners^{465, 466, 468} before processing. Cleaning reduces the bioburden and removes foreign material (i.e., organic residue and inorganic salts) that interferes with the sterilization process by acting as a barrier to the sterilization agent^{179, 426, 457, 911, 912}. Surgical instruments are generally presoaked or prerinsed to prevent drying of blood and tissue. Precleaning in patient-care areas may be needed on items that are heavily soiled with feces, sputum, blood, or other material. Items sent to central processing without removing gross soil may be difficult to clean because of dried secretions and excretions. Cleaning and decontamination should be done as soon as possible after items have been used. Several types of mechanical cleaning machines (e.g., utensil washer-sanitizer, ultrasonic cleaner, washer-sterilizer, dishwasher, washer-disinfector) may facilitate cleaning and decontamination of most items. This equipment often is automated and may increase productivity, improve cleaning effectiveness, and decrease worker exposure to blood and body fluids. Delicate and intricate objects and heat- or moisture-sensitive articles may require careful cleaning by hand. All used items sent to the central processing area should be considered contaminated (unless decontaminated in the area of origin), handled with gloves (forceps or tongs are sometimes needed to avoid exposure to sharps), and decontaminated by one of the aforementioned methods to render them safer to handle. Items composed of more than one removable part should be disassembled. Care should be taken to ensure that all parts are kept together, so that reassembly can be accomplished efficiently⁸¹¹. Investigators have described the degree of cleanliness by visual and microscopic examination. One study found 91% of the instruments to be clean visually but, when examined microscopically, 84% of the instruments had residual debris. Sites that contained residual debris included junctions between insulating

sheaths and activating mechanisms of laparoscopic instruments and articulations and grooves of forceps. More research is needed to understand the clinical significance of these findings 960 and how to ensure proper cleaning. Personnel working in the decontamination area should wear household-cleaning-type rubber or plastic gloves when handling or cleaning contaminated instruments and devices. Face masks, eye protection such as goggles or full-length faceshields, and appropriate gowns should be worn when exposure to blood and contaminated fluids may occur (e.g., when manually cleaning contaminated devices)961. Contaminated instruments are a source of microorganisms that could inoculate personnel through nonintact skin on the hands or through contact with the mucous membranes of eyes, nose, or mouth214, 811, 813. Reusable sharps that have been in contact with blood present a special hazard. Employees must not reach with their gloved hands into trays or containers that hold these sharps to retrieve them214. Rather, employees should use engineering controls (e.g., forceps) to retrieve these devices. Once items are cleaned, dried, and inspected, those requiring sterilization must be wrapped or placed in rigid containers and should be arranged in instrument trays/baskets according to the guidelines provided by the AAMI and other professional organizations454, 811-814, 819, 836, 962. These guidelines state that hinged instruments should be opened; items with removable parts should be disassembled unless the device manufacturer or researchers provide specific instructions or test data to the contrary181; complex instruments should be prepared and sterilized according to device manufacturer's instructions and test data; devices with concave surfaces should be positioned to facilitate drainage of water; heavy items should be positioned not to damage delicate items; and the weight of the instrument set should be based on the design and density of the instruments and the distribution of metal mass811, 962. While there is no longer a specified sterilization weight limit for surgical sets, heavy metal mass is a cause of wet packs (i.e., moisture inside the case and tray after completion of the sterilization cycle)963. Other parameters that may

influence drying are the density of the wraps and the design of the set⁹⁶⁴. There are several choices in methods to maintain sterility of surgical instruments, including rigid containers, peel-open pouches (e.g., self-sealed or heat-sealed plastic and paper pouches), roll stock or reels (i.e., paper-plastic combinations of tubing designed to allow the user to cut and seal the ends to form a pouch) ⁴⁵⁴ and sterilization wraps (woven and nonwoven). Healthcare facilities may use all of these packaging options. The packaging material must allow penetration of the sterilant, provide protection against contact contamination during handling, provide an effective barrier to microbial penetration, and maintain the sterility of the processed item after sterilization ⁹⁶⁵. An ideal sterilization wrap would successfully address barrier effectiveness, penetrability (i.e., allows sterilant to penetrate), aeration (e.g., allows ETO to dissipate), ease of use, drapeability, flexibility, puncture resistance, tear strength, toxicity, odor, waste disposal, linting, cost, and transparency⁹⁶⁶. Unacceptable packaging for use with ETO (e.g., foil, polyvinylchloride, and polyvinylidene chloride [kitchen-type transparent wrap]) ⁸¹⁴ or hydrogen peroxide gas plasma (e.g., linens and paper) should not be used to wrap medical items. In central processing, double wrapping can be done sequentially or nonsequentially (i.e., simultaneous wrapping). Wrapping should be done in such a manner to avoid tenting and gapping. The sequential wrap uses two sheets of the standard sterilization wrap, one wrapped after the other. This procedure creates a package within a package. The nonsequential process uses two sheets wrapped at the same time so that the wrapping needs to be performed only once. This latter method provides multiple layers of protection of surgical instruments from contamination and saves time since wrapping is done only once. Multiple layers are still common practice due to the rigors of handling within the facility even though the barrier efficacy of a single sheet of wrap has improved over the years⁹⁶⁶. Written and illustrated procedures for preparation of items to be packaged should be readily available and used by personnel when packaging procedures are performed⁴⁵⁴. All items to be

sterilized should be arranged so all surfaces will be directly exposed to the sterilizing agent. Thus, loading procedures must allow for free circulation of steam (or another sterilant) around each item. Historically, it was recommended that muslin fabric packs should not exceed the maximal dimensions, weight, and density of 12 inches wide × 12 inches high × 20 inches long, 12 lbs, and 7.2 lbs per cubic foot, respectively. Due to the variety of textiles and metal/plastic containers on the market, the textile and metal/plastic container manufacturer and the sterilizer manufacturers should be consulted for instructions on pack preparation and density parameters⁸¹⁹. There are several important basic principles for loading a sterilizer: allow for proper sterilant circulation; perforated trays should be placed so the tray is parallel to the shelf; nonperforated containers should be placed on their edge (e.g., basins); small items should be loosely placed in wire baskets; and peel packs should be placed on edge in perforated or mesh bottom racks or baskets^{454, 811, 836}. Studies in the early 1970s suggested that wrapped surgical trays remained sterile for varying periods depending on the type of material used to wrap the trays. Safe storage times for sterile packs vary with the porosity of the wrapper and storage conditions (e.g., open versus closed cabinets). Heat-sealed, plastic peel-down pouches and wrapped packs sealed in 3-mil (3/1000 inch) polyethylene overwrap have been reported to be sterile for as long as 9 months after sterilization. The 3-mil polyethylene is applied after sterilization to extend the shelf life for infrequently used items⁹⁶⁷. Supplies wrapped in double-thickness muslin comprising four layers, or equivalent, remain sterile for at least 30 days. Any item that has been sterilized should not be used after the expiration date has been exceeded or if the sterilized package is wet, torn, or punctured. Although some hospitals continue to date every sterilized product and use the time-related shelf-life practice, many hospitals have switched to an event-related shelf-life practice. This latter practice recognizes that the product should remain sterile until some event causes the item to become contaminated (e.g., tear in packaging, packaging becomes

wet, seal is broken)⁹⁶⁸. Event-related factors that contribute to the contamination of a product include bioburden (i.e., the amount of contamination in the environment), air movement, traffic, location, humidity, insects, vermin, flooding, storage area space, open/closed shelving, temperature, and the properties of the wrap material^{966, 969}. There are data that support the event-related shelf-life practice⁹⁷⁰⁻⁹⁷². One study examined the effect of time on the sterile integrity of paper envelopes, peel pouches, and nylon sleeves. The most important finding was the absence of a trend toward an increased rate of contamination over time for any pack when placed in covered storage⁹⁷¹. Another evaluated the effectiveness of event-related outdating by microbiologically testing sterilized items. During the 2-year study period, all of the items tested were sterile⁹⁷². Thus, contamination of a sterile item is event-related and the probability of contamination increases with increased handling⁹⁷³. Following the sterilization process, medical and surgical devices must be handled using aseptic technique in order to prevent contamination. Sterile supplies should be stored far enough from the floor (8 to 10 inches), the ceiling (5 inches unless near a sprinkler head [18 inches from sprinkler head]), and the outside walls (2 inches) to allow for adequate air circulation, ease of cleaning, and compliance with local fire codes (e.g., supplies must be at least 18 inches from sprinkler heads). Medical and surgical supplies should not be stored under sinks or in other locations where they can become wet. Sterile items that become wet are considered contaminated because moisture brings with it microorganisms from the air and surfaces. Closed or covered cabinets are ideal but open shelving may be used for storage. Any package that has fallen or been dropped on the floor must be inspected for damage to the packaging and contents (if the items are breakable). If the package is heat-sealed in impervious plastic and the seal is still intact, the package should be considered not contaminated. If undamaged, items packaged in plastic need not be reprocessed. The sterilization procedure should be monitored routinely by using a combination of mechanical, chemical, and biological

indicators to evaluate the sterilizing conditions and indirectly the microbiologic status of the processed items. The mechanical monitors for steam sterilization include the daily assessment of cycle time and temperature by examining the temperature record chart (or computer printout) and an assessment of pressure via the pressure gauge. The mechanical monitors for ETO include time, temperature, and pressure recorders that provide data via computer printouts, gauges, and/or displays⁸¹⁴. Generally, two essential elements for ETO sterilization (i.e., the gas concentration and humidity) cannot be monitored in healthcare ETO sterilizers. Chemical indicators are convenient, are inexpensive, and indicate that the item has been exposed to the sterilization process. In one study, chemical indicators were more likely than biological indicators to inaccurately indicate sterilization at marginal sterilization times (e.g., 2 minutes)⁸⁴⁷. Chemical indicators should be used in conjunction with biological indicators, but based on current studies should not replace them because they indicate sterilization at marginal sterilization time and because only a biological indicator consisting of resistant spores can measure the microbial killing power of the sterilization process.^{847, 974}. Chemical indicators are affixed on the outside of each pack to show that the package has been processed through a sterilization cycle, but these indicators do not prove sterilization has been achieved. Preferably, a chemical indicator also should be placed on the inside of each pack to verify sterilant penetration. Chemical indicators usually are either heat-or chemical-sensitive inks that change color when one or more sterilization parameters (e.g., steam-time, temperature, and/or saturated steam; ETO-time, temperature, relative humidity and/or ETO concentration) are present. Chemical indicators have been grouped into five classes based on their ability to monitor one or multiple sterilization parameters^{813, 819}. If the internal and/or external indicator suggests inadequate processing, the item should not be used⁸¹⁵. An air-removal test (Bowie-Dick Test) must be performed daily in an empty dynamic-air-removal sterilizer (e.g., prevacuum steam sterilizer) to ensure air removal.

Biological indicators are recognized by most authorities as being closest to the ideal monitors of the sterilization process 974, 975 because they measure the sterilization process directly by using the most resistant microorganisms (i.e., *Bacillus* spores), and not by merely testing the physical and chemical conditions necessary for sterilization. Since the *Bacillus* spores used in biological indicators are more resistant and present in greater numbers than are the common microbial contaminants found on patient-care equipment, the demonstration that the biological indicator has been inactivated strongly implies that other potential pathogens in the load have been killed⁸⁴⁴. An ideal biological monitor of the sterilization process should be easy to use, be inexpensive, not be subject to exogenous contamination, provide positive results as soon as possible after the cycle so that corrective action may be accomplished, and provide positive results only when the sterilization parameters (e.g., steam-time, temperature, and/or saturated steam; ETO-time, temperature, relative humidity and/or ETO concentration) are inadequate to kill microbial contaminants⁸⁴⁷. Biological indicators are the only process indicators that directly monitor the lethality of a given sterilization process. Spores used to monitor a sterilization process have demonstrated resistance to the sterilizing agent and are more resistant than the bioburden found on medical devices^{179, 911, 912}. *B. atrophaeus* spores (106) are used to monitor ETO and dry heat, and *G. stearothermophilus* spores (105) are used to monitor steam sterilization, hydrogen peroxide gas plasma, and liquid peracetic acid sterilizers. *G. stearothermophilus* is incubated at 55-60°C, and *B. atrophaeus* is incubated at 35-37°C. Steam and low temperature sterilizers (e.g., hydrogen peroxide gas plasma, peracetic acid) should be monitored at least weekly with the appropriate commercial preparation of spores. If a sterilizer is used frequently (e.g., several loads per day), daily use of biological indicators allows earlier discovery of equipment malfunctions or procedural errors and thus minimizes the extent of patient surveillance and product recall needed in the event of a positive biological indicator⁸¹¹. Each load should be monitored if it

contains implantable objects. If feasible, implantable items should not be used until the results of spore tests are known to be negative. Originally, spore-strip biological indicators required up to 7 days of incubation to detect viable spores from marginal cycles (i.e., when few spores remained viable). The next generation of biological indicator was self-contained in plastic vials containing a spore-coated paper strip and a growth media in a crushable glass ampoule. This indicator had a maximum incubation of 48 hours but significant failures could be detected in £24 hours. A rapid-readout biological indicator that detects the presence of enzymes of *G. stearothermophilus* by reading a fluorescent product produced by the enzymatic breakdown of a nonfluorescent substrate has been marketed for the more than 10 years. Studies demonstrate that the sensitivity of rapid-readout tests for steam sterilization (1 hour for 132°C gravity sterilizers, 3 hrs for 121°C gravity and 132°C vacuum sterilizers) parallels that of the conventional sterilization-specific biological indicators 846, 847, 976, 977 and the fluorescent rapid readout results reliably predict 24- and 48-hour and 7-day growth⁹⁷⁸. The rapid-readout biological indicator is a dual indicator system as it also detects acid metabolites produced during growth of the *G. stearothermophilus* spores. This system is different from the indicator system consisting of an enzyme system of bacterial origin without spores. Independent comparative data using suboptimal sterilization cycles (e.g., reduced time or temperature) with the enzyme-based indicator system have not been published⁹⁷⁹. A new rapid-readout ETO biological indicator has been designed for rapid and reliable monitoring of ETO sterilization processes. The indicator has been cleared by the FDA for use in the United States⁴⁰⁰. The rapid-readout ETO biological indicator detects the presence of *B. atrophaeus* by detecting a fluorescent signal indicating the activity of an enzyme present within the *B. atrophaeus* organism, beta-glucosidase. The fluorescence indicates the presence of an active spore-associated enzyme and a sterilization process failure. This indicator also detects acid metabolites produced during growth of the *B. atrophaeus* spore. Per

manufacturer's data, the enzyme always was detected whenever viable spores were present. This was expected because the enzyme is relatively ETO resistant and is inactivated at a slightly longer exposure time than the spore. The rapid-readout ETO biological indicator can be used to monitor 100% ETO, and ETO-HCFC mixture sterilization cycles. It has not been tested in ETO-CO₂ mixture sterilization cycles. The standard biological indicator used for monitoring full-cycle steam sterilizers does not provide reliable monitoring flash sterilizers⁹⁸⁰. Biological indicators specifically designed for monitoring flash sterilization are now available, and studies comparing them have been published^{846, 847, 981}. Since sterilization failure can occur (about 1% for steam)⁹⁸², a procedure to follow in the event of positive spore tests with steam sterilization has been provided by CDC and the Association of periOperative Registered Nurses (AORN). The 1981 CDC recommendation is that "objects, other than implantable objects, do not need to be recalled because of a single positive spore test unless the steam sterilizer or the sterilization procedure is defective." The rationale for this recommendation is that single positive spore tests in sterilizers occur sporadically. They may occur for reasons such as slight variation in the resistance of the spores⁹⁸³, improper use of the sterilizer, and laboratory contamination during culture (uncommon with self-contained spore tests). If the mechanical (e.g., time, temperature, pressure in the steam sterilizer) and chemical (internal and/or external) indicators suggest that the sterilizer was functioning properly, a single positive spore test probably does not indicate sterilizer malfunction but the spore test should be repeated immediately ⁹⁸³. If the spore tests remain positive, use of the sterilizer should be discontinued until it is serviced¹. Similarly, AORN states that a single positive spore test does not necessarily indicate a sterilizer failure. If the test is positive, the sterilizer should immediately be rechallenged for proper use and function. Items, other than implantable ones, do not necessarily need to be recalled unless a sterilizer malfunction is found. If a sterilizer malfunction is discovered, the items must be considered nonsterile, and the items from

the suspect load(s) should be recalled, insofar as possible, and reprocessed 984. A suggested protocol for management of positive biological indicators is shown in Table 12839. A more conservative approach also has been recommended 813 in which any positive spore test is assumed to represent sterilizer malfunction and requires that all materials processed in that sterilizer, dating from the sterilization cycle having the last negative biologic indicator to the next cycle showing satisfactory biologic indicator challenge results, must be considered nonsterile and retrieved, if possible, and reprocessed. This more conservative approach should be used for sterilization methods other than steam (e.g., ETO, hydrogen peroxide gas plasma). However, no action is necessary if there is strong evidence for the biological indicator being defective 983 or the growth medium contained a *Bacillus* contaminant 985. If patient-care items were used before retrieval, the infection control professional should assess the risk of infection in collaboration with central processing, surgical services, and risk management staff. The factors that should be considered include the chemical indicator result (e.g., nonreactive chemical indicator may indicate temperature not achieved); the results of other biological indicators that followed the positive biological indicator (e.g., positive on Tuesday, negative on Wednesday); the parameters of the sterilizer associated with the positive biological indicator (e.g., reduced time at correct temperature); the time-temperature chart (or printout); and the microbial load associated with decontaminated surgical instruments (e.g., 85% of decontaminated surgical instruments have less than 100 CFU). The margin of safety in steam sterilization is sufficiently large that there is minimal infection risk associated with items in a load that show spore growth, especially if the item was properly cleaned and the temperature was achieved (e.g., as shown by acceptable chemical indicator or temperature chart). There are no published studies that document disease transmission via a nonretrieved surgical instrument following a sterilization cycle with a positive biological indicator. False-positive biological indicators may occur from improper testing

or faulty indicators. The latter may occur from improper storage, processing, product contamination, material failure, or variation in resistance of spores. Gram stain and subculture of a positive biological indicator may determine if a contaminant has created a false-positive result^{839, 986}. However, in one incident, the broth used as growth medium contained a contaminant, *B. coagulans*, which resulted in broth turbidity at 55°C⁹⁸⁵. Testing of paired biological indicators from different manufacturers can assist in assessing a product defect⁸³⁹. False-positive biological indicators due to extrinsic contamination when using self-contained biological indicators should be uncommon. A biological indicator should not be considered a false-positive indicator until a thorough analysis of the entire sterilization process shows this to be likely. The size and composition of the biological indicator test pack should be standardized to create a significant challenge to air removal and sterilant penetration and to obtain interpretable results. There is a standard 16-towel pack recommended by AAMI for steam sterilization^{813, 819, 987} consisting of 16 clean, preconditioned, reusable huck or absorbent surgical towels each of which is approximately 16 inches by 26 inches. Each towel is folded lengthwise into thirds and then folded widthwise in the middle. One or more biological indicators are placed between the eight and ninth towels in the approximate geometric center of the pack. When the towels are folded and placed one on top of another, to form a stack (approximately 6 inch height) it should weigh approximately 3 pounds and should have a density of approximately 11.3 pounds per cubic foot⁸¹³. This test pack has not gained universal use as a standard pack that simulates the actual in-use conditions of steam sterilizers. Commercially available disposable test packs that have been shown to be equivalent to the AAMI 16 towel test pack also may be used. The test pack should be placed flat in an otherwise fully loaded sterilizer chamber, in the area least favorable to sterilization (i.e., the area representing the greatest challenge to the biological indicator). This area is normally in the front, bottom section of the sterilizer, near the drain^{811, 813}. A control biological indicator from the lot used

for testing should be left unexposed to the sterilant, and then incubated to verify the presterilization viability of the test spores and proper incubation. The most conservative approach would be to use a control for each run; however, less frequent use may be adequate (e.g., weekly). There also is a routine test pack for ETO where a biological indicator is placed in a plastic syringe with plunger, then placed in the folds of a clean surgical towel, and wrapped. Alternatively, commercially available disposal test packs that have been shown to be equivalent to the AAMI test pack may be used. The test pack is placed in the center of the sterilizer load⁸¹⁴. Sterilization records (mechanical, chemical, and biological) should be retained for a time period in compliance with standards (e.g., Joint Commission for the Accreditation of Healthcare Facilities requests 3 years) and state and federal regulations. In Europe, biological monitors are not used routinely to monitor the sterilization process. Instead, release of sterilizer items is based on monitoring the physical conditions of the sterilization process that is termed "parametric release." Parametric release requires that there is a defined quality system in place at the facility performing the sterilization and that the sterilization process be validated for the items being sterilized. At present in Europe, parametric release is accepted for steam, dry heat, and ionizing radiation processes, as the physical conditions are understood and can be monitored directly⁹⁸⁸. For example, with steam sterilizers the load could be monitored with probes that would yield data on temperature, time, and humidity at representative locations in the chamber and compared to the specifications developed during the validation process. Periodic infection control rounds to areas using sterilizers to standardize the sterilizer's use may identify correctable variances in operator competence; documentation of sterilization records, including chemical and biological indicator test results; sterilizer maintenance and wrapping; and load numbering of packs. These rounds also may identify improvement activities to ensure that operators are adhering to established standards.⁹⁸⁹ CDC provides information on infection control and clinical safety to help

reduce the risk of infections among healthcare workers, patients, and visitors.

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