REVIEW ARTICLE

Environmental sampling of hospital surfaces: Assessing methodological quality

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ABSTRACT

Background: Patients in rooms previously occupied by individuals with antimicrobial-resistant organisms are at an increased risk of infection. To combat this risk, environmental methods such as self-disinfecting surfaces, ultraviolet light, and titanium dioxide paint are entering the clinical setting to supplement traditional prevention methods. The advent of these novel technologies for infection control and prevention necessitates a standardized method of assessing environmental surface bioburden; however, there is currently no standardized protocol for sampling hard, non-porous surfaces.

Objectives: This article reviews the literature for environmental sampling methodologies and assesses them for rigor and appropriateness. This review and its assessment tool aim to guide a clinical audience in assessing the methodological integrity of study protocols, including collection, transportation, recovery, and culturing of environmental surface samples.

Methods: A search of PubMed and MEDLINE was performed and 122 articles and their references were reviewed.

Results: Environmental sampling methods include elution-dependent (pre-moistened swabs, sponges, wipes) and elution-independent methods (Replicate Organism Detection and Counting plates, 3M Petrifilm™ plates, dipslides). With both methods, moisture and neutralizers must be present at the time of sampling to increase recovery rates. Elution-dependent methods also require physical dissociation methods to release organisms from the collection device prior to culturing. Furthermore, special consideration is needed for the collection, recovery, and culturing of spore-forming organisms.

Conclusions: Standardization of environmental surface sampling methods in the collection, transportation, recovery, and culture of a microbial sample is needed to objectively assess and compare the efficacy of newer antimicrobial technologies.

KEYWORDS
Environmental sampling; organism recovery; collection; transport; methodology; plating

INTRODUCTION

The relative importance of environmental contamination in hospital-acquired infections is still debated; however, it is clear that patients in rooms previously occupied by individuals with antimicrobial-resistant organisms are at increased risk of colonization or infection with these same microbes [1]. Reducing the microbial burden in healthcare environments decreases the transmission of microorganisms; therefore, an increasing number of novel adjunctive technologies to supplement routine cleaning and disinfection are being developed. These include new disinfection technologies such as ultraviolet (UV) light disinfection, ozonated water, and self-disinfecting surfaces such as copper-alloy materials and titanium dioxide paints [2-4]. An assessment of antimicrobial efficacy is essential in the evaluation of these products. However, testing methodologies vary significantly in current literature due to the lack of standardization by regulatory bodies [3, 5]. Consequently, this poses a challenge to the infection preventionist when evaluating product performance.

This review summarizes the key steps in the a) collection, b) transport, c) recovery, and d) culture processing steps that should be outlined by environmental sampling studies for microorganisms. We expand on a previous review article by Galvin et al. (2012) [6] describing microbial monitoring methods of hospital environments by including an environmental sampling methodologic quality assessment tool (Figure 1),

Acknowledgements: None.
Conflicts of interest: None.
Funding: This article was funded by the Vancouver General Hospital and University of British Columbia Hospital Foundation.

comparison tables for specimen collection, recovery, and culturing methods (Tables 1 and 2), special considerations for clostridial spores, and information on current environmental sampling standards.

Our aim is to assist infection preventionists and clinicians in understanding the sampling methodology in order to 1) assess the quality of study results and 2) guide those who are considering performing an in-house assessment of a product.

METHODS

Articles related to environmental sampling of vegetative bacteria and spores on non-porous, solid surfaces (stainless steel, metal, glass, ceramic, painted or coated wood, plastic) were sought through both PubMed and MEDLINE with the following keywords: recovery method, environmental sampling, bacteria, spores, and non-porous surface. The abstracts and references of 122 articles were reviewed and, as part of this narrative review, 98 were selected as relevant to the theme of environmental sampling methods. Methodology was then assessed for applicability to healthcare. In addition, guidelines from the Centers for Disease Control and Prevention, the American Society for Testing and Materials (ASTM), and the International Organization for Standardization (ISO) were reviewed. Searches were limited to the English language and no limits were placed on publication dates. Adenosine Triphosphate bioluminescence testing was excluded due to its limited role as a research tool for environmental sampling despite its practical role in assessing hospital surface cleanliness following disinfectant use.

RESULTS AND DISCUSSION

Specimen collection

The most common surfaces evaluated are non-porous, including high-touch hospital surfaces such as bed rails, tabletops, and arm rests. Elution-dependent methods (swabs, sponges, and wipes) and elution-independent methods (contact plates, dipslides, Petrifilm™ plates [3M, St. Paul, MN]) are appropriate for these surfaces. Porous surfaces generally comprise textiles and, in these cases, vacuum filter socks and microvacuums, and bulk sampling methods are most appropriate [7].

1. Specimen collection for elution-dependent methods

Elution refers to the immersion of the collection device in an eluent and the use of a physical dissociation method such as shaking, sonicating, vortexing, or stomaching to recover the microorganisms. Swabs are most commonly used for regular or irregularly shaped smaller surfaces, typically between 20 cm^2 and 100 cm^2, including hard-to-reach areas such as corners, bed rails, and crevices [8, 9]. Both swab tip and shaft compositions should be reported due to their effect on recovery efficiencies (e.g., cotton swab with a wooden shaft) [8]. Cotton and calcium alginate swab buds, in particular, tend to underestimate the amount of microbial contamination in comparison to other swab buds, including rayon, macrofoam, nylon, and polyester [8, 10, 11]. The swab shaft also plays a critical role in determining the amount of mechanical energy placed on the swab bud, as more rigid materials increase recovery [8].

Swabbing technique should be specified, including the sample area, angle of swabbing, portion of swab used, swabbing duration, swabbing direction (e.g., vertical, horizontal, diagonal), strokes in each direction, and number of swabs used for each sample. A set area should also be delineated with a corrosion-resistant template that can be sterilized or replaced between swabs [9, 12]. Prior to swabbing, it is important that the swab bud be pressed against the side of the tube to standardize the volume of pre-moistening liquid in each swab. Consistency with degree of pressure and the speed of swabbing can be improved by having one investigator perform all of the sampling. In addition, one study proposes the use of two sequential swabs to increase recovery [13]. A proposed angle of sampling is 30 degrees, where swabs are rotated 120 degrees when the direction is changed from horizontal to vertical and then to a diagonal sampling pattern [14].

Sponges and wipes are generally used for sampling larger regular or irregularly shaped (100 cm^2 to 1 m^2) surface areas such as walls and floors [7, 9, 15]. They are usually made from rayon, polyester, cellulose, polyurethane, or cotton, although studies comparing recovery among these different materials are limited. Sponges may allow for better recovery of pathogens compared to swabs due to the larger surface area sampled [16]. A suggested standardization method includes sampling horizontally, vertically, and then diagonally, noting the strokes per direction while turning to reveal a new surface with each new direction [7, 14].

2. Specimen collection for elution-independent methods

Agar contact methods include contact plates such as Replicate Organism Detection and Counting (RODAC) contact plates, Petrifilm™ plates, and dipslides. They are limited to use on smaller surfaces: usually between 20-26 cm^2 for RODAC and Petrifilm™ plates or 7-12 cm^2 for dipslides [7, 9]. RODAC plates and dipslides must be used on smooth, flat, non-porous surfaces; however, due to their flexibility, Petrifilm™ plates can be used on irregularly shaped surfaces such as door handles [17]. The agar plate should be pressed firmly onto the surface for a standardized amount of time and pressure. ISO Standard 18593 for environmental sampling of food industry environments (Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs) recommends ten seconds at 500 g, although other studies have used different pressure (840 g) or different times (30 seconds) [9, 18, 19].

3. Pre-moistening fluid, eluents, and neutralizers

Sampling environmental surfaces requires that moisture be present either on the surface or through pre-moistened swabs, wipes, sponges, and agar plates to minimize microbial desiccation and enhance spore recovery [8]. Eluents or rinse fluids (phosphate buffered saline [PBS], buffered or unbuffered peptone water, and ringer solutions) are often used as pre-moistening liquids [7, 20]. However, environmental surfaces in hospitals usually contain disinfectant residues such as quaternary ammonium compounds, hydrogen peroxide, phenolics, and sodium hypochlorites that may inhibit microbial growth and/or identification upon subsequent culture. Therefore, neutralizing agents are also required at collection time to counteract the effects of all disinfectant residues, except for vaporized hydrogen peroxide, whose end products are oxygen and water [7, 12, 21]. Common neutralizers include lecithin and polysorbate (Tween) 80, Dey Engley (D/E) broth or agar, sodium thiosulfate, glycine, and catalase [12]. Selection should also be based on disinfectant used, compatibility with desired assays, and toxicity to the desired microbe. In addition, if enumeration is intended, enrichment ingredients such as Trypticase soy broth or Brain Heart Infusion broth should not be added [6]. However, if identification of specific bacteria is required – for an outbreak investigation, for example – enrichment can be considered. It is important to note that some eluents such as PBS may hinder microbial recovery through salt crystal precipitation on metal surfaces [14]. Similarly, some neutralizers may have inhibitory effects, including sodium thiosulfate and D/E on some *Staphylococci* species and mycobacteria species, respectively [21, 22].

Like elution-dependent methods, moistened media and neutralizers are needed within the agar to improve recovery, increase bacterial clump dispersion, and minimize desiccation or residual effects of disinfectants [23]. In addition, direct contact agar methods can only be used on surfaces that contain low amounts of microorganisms to avoid a confluence of growth and underestimation of bioburden [10, 23]. This method does not detect dormant or sub-lethally damaged organisms, including those that are viable but non-culturable [10].

Table 1 summarizes the advantages and limitations of elution-dependent and -independent methods.

TABLE 1: Advantages and limitations of collection methods for non-porous surfaces.

Collection Method	Standard	Sampling Location	Advantages	Limitations
Elution-dependent				
Swabs Swab bud: Cotton, calcium alginate, flocked nylon, polyester, macrofoam, polyurethane, rayon Swab shaft: Wood, aluminum, polypropylene, polystyrene	Food industry: Yes Healthcare: No	Small surfaces (20-100 cm^2) [9]: regular or irregularly shaped surfaces (doorknobs, keyboards, corners, crevices)	Results may be better than contact plates for G-organisms [18]	a. *Personnel*: Difficult to standardize pressure, speed; error with pipetting or diluting b. *Material* affects absorption and release of organisms c. *Nature of surface*: Disinfectant residues, biofilm reduce recovery [10] d. *Swab shaft* determines mechanical energy placed onto swab bud [8]
Sponges or wipes Rayon, polyester, cellulose, polyurethane, cotton	Food industry: Yes Healthcare: No	Large surfaces (100 cm^2-1 m^2) [7, 9]: walls, floors, countertops; regular or irregularly shaped surfaces	Can sample multiple sites [16]	e. *Drying effects* of wipes reduce collection over large areas [15]
Elution-independent				
RODAC plates Media Non-selective agar Selective agar	Food industry: Yes Healthcare: No	Regularly shaped (smooth, flat) surface only *Size*: Agar plate surface area (SA) (around 20-26 cm^2) [7]	Time-efficient; no processing needed	a. *Personnel*: Hard to standardize contact time and pressure b. *Nature of plating*: Limited by surface area of contact plate Only for low number of bacteria as dilution cannot be performed [10] Excludes sub-lethally damaged and dormant bacteria Coalescence of colonies underestimates colony-forming unit [10] c. *Nature of surface*: Full contact with surface needed
3M Petrifilm™ Media Thin, dehydrated proprietary media located between two films	Food industry: No Healthcare: No	Regular or irregularly shaped surfaces *Size*: Petrifilm™ SA (around 25 cm^2) [7, 17]	No processing; less incubator space needed; flexibility around irregularly shaped surfaces	
Commercial dipslides Media Non-selective agar Selective agar	Food industry: No Healthcare: No	Regularly shaped (flat, smooth) surfaces only *Size*: Dipslide SA (around 7-12 cm^2) [7, 9]	No processing needed	

Transport and storage

The sample must be transported for laboratory analysis ideally within four hours [9]. Storing samples at 1°C to 8°C is generally recommended, especially if more than 24 hours' transport is anticipated [9, 24]. If shipping is required, an additional container should be used around the sample container to minimize the impact of temperature and/or altitude fluctuations.

Recovery methods

Recovery methods refer to the process of extracting microorganisms from the collection device. Swabs, sponges, and wipes should never be directly subcultured onto solid media. Rather, physical dissociation methods (PDM) are necessary to separate bacterial aggregates and allow for a more representative microbial count similar to the original bioburden. PDM include manual or mechanical shaking (vortexing), sonicating, or stomaching to release the bacteria (Table 2) [7].

Manually shaking swab containers and massaging sponge and wipe bags produce variable results that are operator- and time-dependent. ISO Standard 18593 recommends the use of a mechanical shaker for swabs and a stomacher (peristaltic homogenizer) for sponges [9]. Laboratory vortex mixers are commonly used but are limited to smaller collection devices and vials of liquid. Platform shakers are also available, although they may provide less mechanical agitation compared to vortexing, resulting in less microorganism recovery [25].

Bacterial sonication can be used to kill or declump bacteria depending on the frequency and duration; lower frequencies are preferred for bacterial declumping of environmental samples. Declumping bacteria ensures better recovery. A thorough article will specifically state the sonicator manufacturer, model number, frequency, and sonication time, though ideally output power, fluid temperature, and reaction volume will be included as well [26]. Generally, studies use low

TABLE 2: Advantages and limitations of recovery and culturing methods.

Recovery Method	Collection Method	Advantages	Limitations
Elution-independent Methods			
Direct plating Contact plating	Swabs Contact plates Petrifilm™ Dipslides	No processing needed See Table 1	Inaccurate enumeration method See Table 1
Elution-dependent Methods			
Spread plate method	Swabs, sponges, wipes	Fewer microorganisms required than pour plating	Operator-dependent (e.g., pressure, time)
Pour plate method	Swabs, sponges, wipes	Better sampling efficiency compared to spread plate [8] Method suitable for anaerobic bacteria	Subsurface colonies difficult to retrieve Thermal shock and dilutional factor from melted agar reduce organism count
Drop plate method	Swabs, sponges, wipes	Less time required to drop samples than to spread Less equipment needed (four dilutions plated on one plate)	Unstandardized methodology (dilutions and volume plated) [31] Only works for pure cultures [31]
Membrane filtration method	Swabs, sponges, wipes	Suspected disinfectant residues can be rinsed multiple times Can be used with large volume rinsates	Labour-intensive Chemicals or particles on membrane may inhibit growth of organism
Physical Dissociation Methods			
Manual shaking	Swabs, wipes	No equipment needed	Labour-intensive Operator-dependent (strength, fatigue)
Bag massaging	Sponges, wipes		Limited to smaller items that fit in a test tube, flask, or beaker
Vortex	Swabs, wipes	Allows for standardization	Limited to smaller items that fit in a test tube, flask, or beaker
Sonication	Swabs, wipes	Allows for standardization (power, frequency, temperature, reaction volume, time)	Higher frequencies or direct probe sonicators may kill microorganisms Cost of sonicator
Stomaching	Sponges, wipes	Allows for standardization	Only for soft items that will not puncture the bag Cost of sonicator

ultrasonic bath frequencies (around 20-40 kHz) to declump bacteria since baths have less potential to inactivate bacteria compared to direct probes [26, 27]. Sonication has been used more frequently in biofilm prostheses, where an ideal sonication time of one to five minutes is used to declump and dislodge bacteria from surfaces; however, this method is rarely used in environmental sampling [28].

Stomaching occurs when the bag containing the collection device and rinse fluids is placed inside a machine, pounded by paddles, and exposed to compression and shearing to remove the bacteria from the collecting device [29]. This method is more appropriate for softer, larger materials such as wipes, gauze pads, and sponges. Manual stomaching is not recommended due to the increased variability of operator force.

Culture plating methods

Eluents are often serially diluted following PDM of the microorganism sample and prior to plating to achieve countable colonies. The ASTM Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces recommends standard spread plate, pour plate, and membrane filtration techniques [30].

Spread plating disperses the rinse fluid onto an agar plate with a sterile spreader and is relatively easy to perform [12]. Pour plating mixes an aliquot of the rinse fluid with molten agar medium. Although pour plating has higher sampling efficiencies than spread plating, colonies exhibit slower growth and a higher bacteria inoculum is required due to the extra dilution factor from the agar medium [8]. The membrane filtration method filters the eluent and microorganism through a membrane filter and then rinses the membrane filter with eluents containing neutralizers if disinfectant residues are suspected. The filter, now containing microorganisms, is then placed onto the agar medium and incubated. This technique is appropriate for large-volume rinsates, low microorganism numbers, and when toxic residues have not been adequately neutralized [12]. Drop plating involves placing drops of different sample dilutions onto each of the four quadrants of an agar plate but requires pure cultures because it cannot distinguish microorganisms in polymicrobial samples [31]. These methods differ in the maximum volume that can be used except for membrane filtration, which has flexible volumes: spread plate 0.1 ml; pour plate 0.5-3 ml; and drop plate 0.1-0.2 ml [12, 31]. Table 2 shows the advantages and disadvantages of each method to help guide selection.

Assessing environmental sampling studies

It is important that each step in the method has been appropriately selected to reflect the study design. Collection, recovery, transport, and culturing methods should be chosen based on the given organism, surface type (porosity, composition), surface size, and location (see Table 2). This also includes a critical analysis of whether elution-dependent or -independent methods should be used. Figure 1 presents an assessment tool for clinicians and infection preventionists when evaluating articles utilizing environmental sampling methods.

There are situations in which one method may be more ideal than others, with surface size and location being key considerations. Swabs are usually used for areas from 20 cm^2 to 100 cm^2; sponges for areas from 100 cm^2 to 1 m^2; Petrifilm™ and RODAC plates for areas from 20 cm^2 to 26 cm^2; and dipslides for areas from 7 cm^2 to 12 cm^2 [7, 8, 9]. Swabs, sponges, and Petrifilm™ plates can be used for regular and irregular surfaces, including hard-to-reach areas, whereas contact plates and dipslides require a flat surface. Qualitative assays, including outbreak investigations, usually require larger surfaces to be investigated; therefore, sponges and wipes may be a good option. Quantitative assays require sampling of specific sites and thus swabs, contact plates (usually non-selective), Petrifilm™ plates, and dipslides can be considered.

The swab composition favoured by most researchers includes macrofoam, flocked nylon, rayon, or polyester. Flocked nylon swabs, a newer technology, have demonstrated the ability to release microorganisms more rapidly and completely, with one study demonstrating 92% release capacity compared to 21% with rayon swabs [32].

Selection of elution-independent methods are equally challenging due to the lack of comparison articles. Two studies, an in-vitro and a clinical study, found Petrifilm™ plates to be more effective than RODAC plates in increasing colony-forming unit detection, except for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) on stainless steel surfaces [17, 33]. Dipslides were shown in one study to be more sensitive than contact plates in detecting MRSA [34].

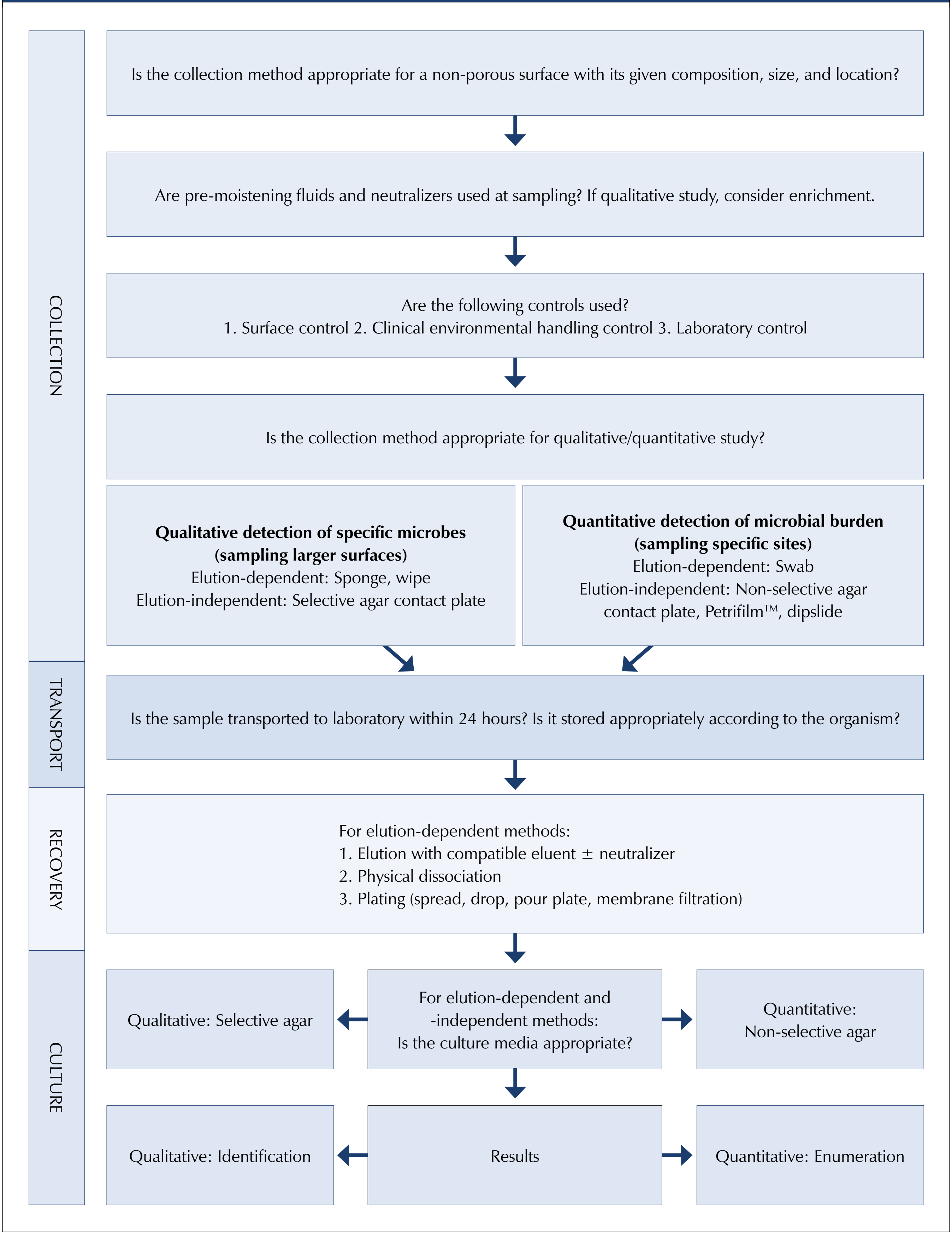
Regardless of the collection method, a moistened collecting device must be used at collection time to improve recovery rates. Appropriate neutralizers must be added and should be selected depending on the disinfectant used. Qualitative studies should be enriched with broth media or use selective agar; quantitative studies should not be enriched and should use non-selective agar.

Importantly, any variable that can impact microorganism recovery requires its own control. At minimum, a surface control, a clinical environmental handling control, and a laboratory control should be used. A surface control is used to compare the results of a sampled surface to a control surface. A clinical environment handling control is used to detect contamination from sample handling by removing the collecting device from its sterile packaging and exposing it to the environment without sampling the surface [7]. A laboratory negative control of unused samples should be standard.

Special considerations for clostridial spores

Environmental sampling of clostridial spores is difficult due to limitations by low sensitivities, anaerobic culture conditions, and extended incubation periods [35]. Spores have been sampled in studies using pre-moistened swabs with or without broth, sponges, and contact plates. In general, sponges and contact plates have been shown to have higher recovery efficacy than swabs [16, 36, 37]. Increased recovery has been

FIGURE 1: Assessment tool for environmental sampling methodologic quality.



shown when using lysozyme or bile salts such as sodium taurocholate and cholic acid in combination with Cycloserine-Cefoxitin Fructose Agar or its broth equivalent [36, 38]. One study demonstrated that broth is better for spore recovery than agar, especially in environmental swabbing, where there are fewer spores than in fecal samples [39]. The use of alkaline thioglycolate as pre-exposure to sensitize spores to lysozyme effects is controversial, with one study demonstrating no difference [38] and two studies demonstrating better recovery, particularly for heat- or alkali-treated spores [35, 40]. Newer media, including *C. difficile* brucella broth with thioglycolic acid and L-cystine, has not yet been extensively peer-reviewed, although their potential advantage is the ability to be incubated in routine clinical laboratory atmospheres.

CONCLUSION

The lack of environmental sampling standardization in healthcare hinders the ability to objectively assess and compare the quality of articles evaluating the efficacy of newer antimicrobial technologies. This variability needs to be addressed by regulatory agencies. The many variables in each of the four process steps (collection, transport, recovery, and culture) can independently influence the quality of the sampling methods and inter-study comparisons are thus admittedly difficult. It is tempting to suggest a limited number of environmental sampling methods to facilitate standardization. Unfortunately, this is a challenge specifically because the selection of each method within the four process steps depends upon the surface, its size, shape, and location, and the results desired (qualitative versus quantitative). In the interim, this article and its assessment tool will hopefully help readers assess the methodologic quality of environmental sampling in healthcare facilities. At a minimum, a description of methodology should consider these elements: 1) moisture must be present at the time of sampling, 2) a neutralizing solution is necessary to arrest residual disinfectant action, 3) a physical dissociation method must be used to release organisms from the collection device prior to culturing, and 4) special consideration is required for the collection and culturing of spore-forming organisms.

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