SAMPLING THE HOSPITAL ENVIRONMENT – A PRACTICAL GUIDE

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THE SURFACE ENVIRONMENT – AN OVERLOOKED VEHICLE FOR INFECTION TRANSMISSION

- Previously, the hospital surface environment was not believed to be an important factor in the transmission of organisms. Maki (1982) stated that the inanimate environment contributed negligibly to HCAI.
- Spaulding (1968) classified surfaces within bed spaces as non-critical as they do not come in contact with broken skin.
- Now, it is becoming clear that surfaces play an important role in the transmission and acquisition of organisms related to healthcare-associated infections.
- It has been proven that not only can pathogenic organisms survive long term on these surfaces, but that there is direct transfer between patients and surfaces.
- Now, literature is being published on these surface environments, looking into the contamination that can be found and how it moves between surfaces.
- Despite these considerations, the hospital environment is only assessed by visual inspection and there is no law or legislation that requires microbiological sampling.

WHAT HAS BEEN ISOLATED FROM THE HOSPITAL SURFACE ENVIRONMENT?

Acinetobacter anitratus	*Acinetobacter baumannii*	*Acinetobacter calcoaceticus*	Carbapenem-resistant *Acinetobacter*	*Acinetobacter junii*
Acinetobacter lwoffii	*Acinetobacter spp.*	Adenovirus	*Alcaligenes faecalis*	*Alcaligenes* spp.
Aspergillus spp.	*Bacillus cereus*	*Bacillus* spp.	*Burkholderia* spp.	*Citrobacter* spp.
Clostridium difficile	*Corynebacterium* spp.	Cytomegalovirus	Carbapenem-resistant *Enterobacteriaceae*	Extended spectrum beta-lactamase producing *Enterobacteriaceae*
Enterococcus faecalis	Vancomycin-resistant *enterococci*	*Enterococcus* spp.	Vancomycin-susceptible *enterococci*	*Enterobacter agglomerans*
Enterobacter spp.	*Escherichia coli*	Extended spectrum beta-lactamase producing *Escherichia coli*	Gram-negative (unspecified)	Gram-positive (unspecified)
Influenza A virus (RNA)	*Klebsiella pneumoniae*	*Micrococcus* spp.	Mimivirus	*Mycoplasma pneumonias*
Panotea spp.	*Proteus* spp.	*Pseudomonas aeruginosa*	*Pseudomonas alcaligenes*	*Pseudomonas putida*
Pseudomonas spp.	*Pseudomonas stutzeri*	Respiratory syncytial virus	Rotavirus	*Sphingomonas paucimobilis*
Unidentified spore former	Coagulase-negative *Staphylococci*	*Staphylococcus arlettae*	*Staphylococcus aureus*	Methicillin-resistant *staphylococcus aureus*
Norovirus	Vancomycin-resistant *Staphylococcus aureus*	*Serratia* spp.	*Staphylococcus cohnii*	*Staphylococcus epidermidis*
Staphylococcus haemolyticus	*Staphylococcus pasteuri*	*Staphylococcus saprohyticus*	*Staphylococcus sciuri*	*Staphylococcus simulans*
Staphylococcus spp.	*Staphylococcus warneri*	*Staphylococcus xilosus*	*Stenotrophomonas maltophilia*	*Streptococcus*
Streptococcus viridians	Torque-teno virus			

ENVIRONMENTAL SURVIVAL

Organism	Infectious Dose (if known)	Length of Survival on Surfaces
Staphylococcus aureus	<15Colony Forming Unit/10^6 (oral dose)	7 days – >1 year
Clostridium difficile	1CFU (in mouse models)	5 months
***Klebsiella* spp.**	No experimental evidence	<1 hour – 30 months
E. coli	10 CFU	<1 hour – 16 months
***Acinetobacter* spp.**	No experimental evidence	3 days - 5 months
Adenovirus	<150 viral copies	7 days – 3 months
Norovirus	10 – 100 viral copies	Norovirus (including Feline Calicvirus) 8 hours – 14 days
Pseudomonas aeruginosa	10^8 (oral dose)	6 hours – 16 months
VRE	No experimental evidence	5 days – 4 months

SAMPLING THE HOSPITAL ENVIRONMENT – WHY?

- Response to outbreak.
- Monitoring for a specific organism.
- Background environmental monitoring.
- Cleaning validations.
- Commission of new environment such as new buildings.

SAMPLING OPTIONS

DIRECT CONTACT METHODS

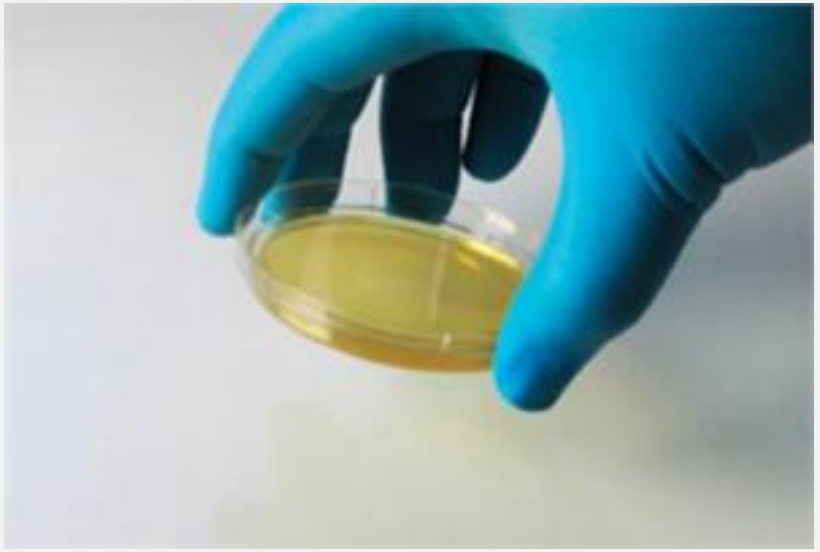
- Contact plate.
- Dipslide.
- Petrifilms.

METHODS REQUIRING EXTRACTION

- Swabs.
- Sponge.
- Wipe methods.

CONTACT PLATES

- Contact plates are discs of agar contained within a petri dish. They are a direct contact method.
- They can be made with selective or non-selective agars.
- The lid is removed and the contact plate is pressed firmly to the test surface. Firm pressure is give for 10 seconds, then the lid is replaced and the contact plate is ready for incubation.



CONTACT PLATES

PROS

- Easy to use.
- Reproducible surface area.
- Selective or non-selective agars.
- Addition of neutralisers.
- Enclosed method; no processing losses.
- Quantification by colony counting.
- Works well for cells adsorbed to a surface.

CONS

- Surface must be flat and even.
- Pressure variable between technicians.
- Do not work well in heavily contaminated surfaces due to clumping of cells.
- Less sensitive than swabs.
- No enrichment process for stressed or damaged cells.
- Recovery variabilities between brands.

DIPSLIDES

- They have two sides which can contain different selective or non-selective agars.
- Unlike contact plates which are unsuitable for uneven surfaces, dipslides have greater flexibility.
- Dipslides are used similarly to contact plates, and are pressed against the test surface for ten seconds. It is then placed back into the container and incubated.

DIPSLIDES

PROS

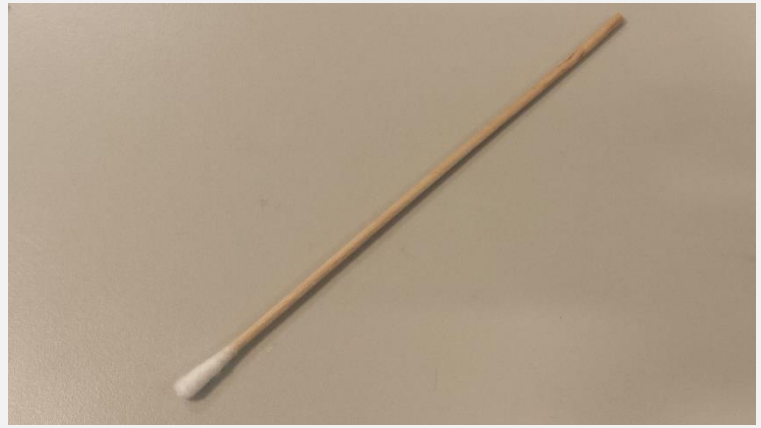
- Similar to contact plates with the addition of;
- Increased flexibility allows sampling of uneven surfaces.
- Two sides can give two samples, and each side can contain different agar, option of personalising dipslide agars for specific function.
- Multifunctional; can be used for liquid testing and inoculation following swabbing.
- Simple basic analysis available following a percentage coverage dipslide chart to give estimation of CFU/cm2 surface contamination.
- Dipslide comparator app for very basic analysis.

CONS

- See contact plates

SWABS

- Swabs can be made of different materials such as cotton, rayon, polyester. Handles can be either wooden or plastic.
- A sample is taken by wetting the swab tip in a sterile solution.
- When swabbing enough pressure should be exerted that the shaft flexes.
- Care is taken to touch only the handle of the swab and not contaminating the tip. The test area is within a 10cm x 10cm square, which can either be visualised or for better reproducibility, with a sterilised guideline.
- Ten firm horizontal strokes are taken with the swab within the test area, while rolling the swab tip on the surface, followed by 10 vertical strokes.



SWABS

PROS

- Can sample uneven surfaces and crevices.
- Easy to exert pressure on surface and recover organisms from biofilm.
- Cheap and often readily available.
- Choice of wetting agent and transport medium for method optimisation.
- Can use direct inoculation or enrichment methods for increase sensitivity.
- Processing for analysis by molecular methods.
- Shown to be effective for recovery of MRSA from surfaces.

CONS

- Processing losses and variability in recovery following processing choices.
- Variable sampling pressure and tip rotation between technicians.
- Variable surface area, unless using sterile coupon guideline.
- Cost of processing.
- Skill required for result interpretation.

SPONGES

- Sponges can be ordered separately, or as a sponge-stick, dry or pre-moistened.
- The sponge is removed from the sterile packaging and a 12x12 inch surface swabbed using pressure enough to cause flex on the stick.
- Area is sampled horizontally. The sponge is turned over, and the same area sampled vertically. The sides of the sponge are used to same diagonally.
- The sponge is then inserted into a sterile bag and is then transported for further processing.

SPONGES

PROS

- Can sample large surface areas easily.
- Can be manipulated around uneven surfaces.
- Cheap.
- Literature suggests sponge sampling methods are the most effective when trying to recover *C. difficile* from surfaces.
- Can use enrichment methods.

CONS

- Potential technician contamination; loose sponges without stick can be easily contaminated.
- Post-test processing losses.

WIPE METHODS

- Wipe methods involve using a piece of sterile cloth or gauze, and wiping the surface using aseptic technique to collect a sample.
- Following a similar methods to sponge sampling, the cloth or gauze is folded over to each time reveal a sterile section for sampling
- The wipe is then aseptically processed to extract the sample.

WIPE METHODS

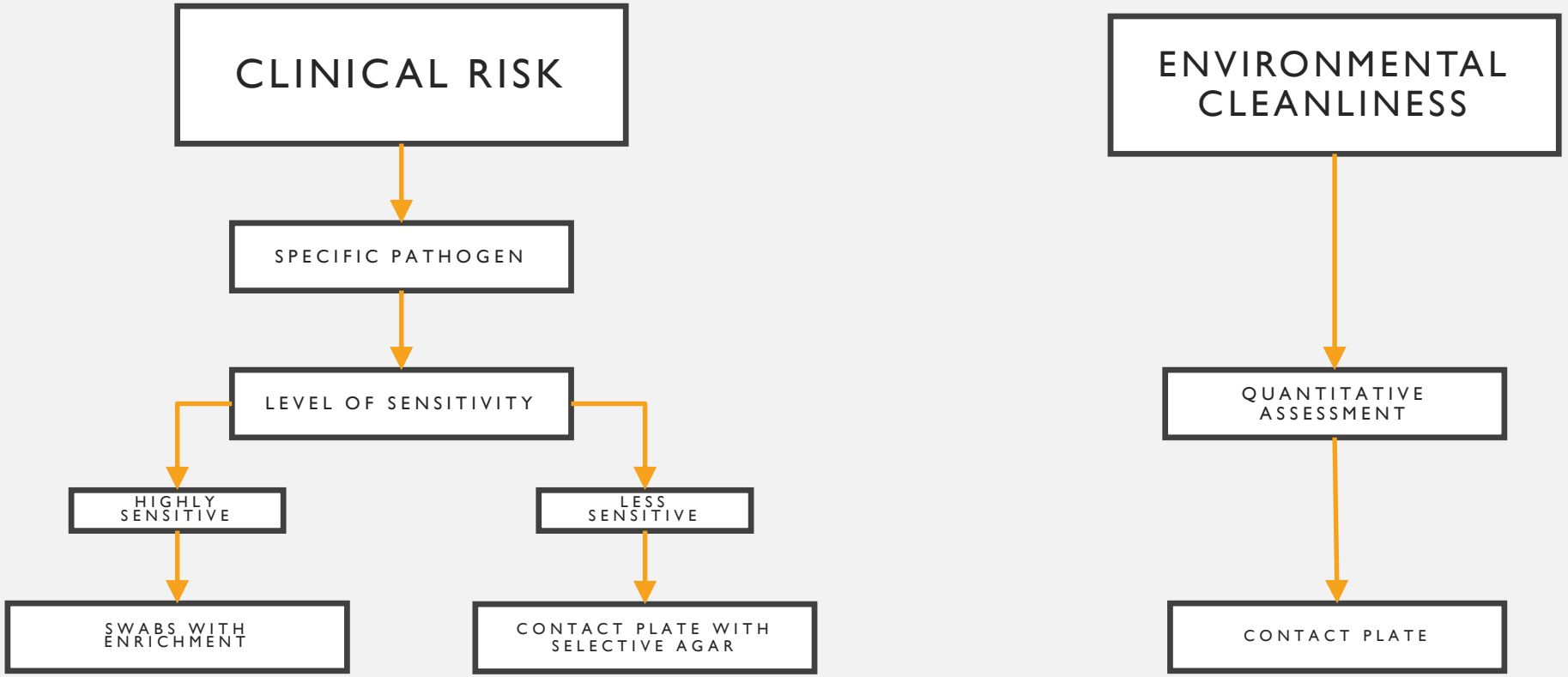
PROS

- Cheap and often readily available.
- Very simple technique.
- Can sample large areas with ease.
- Can use enrichment methods.

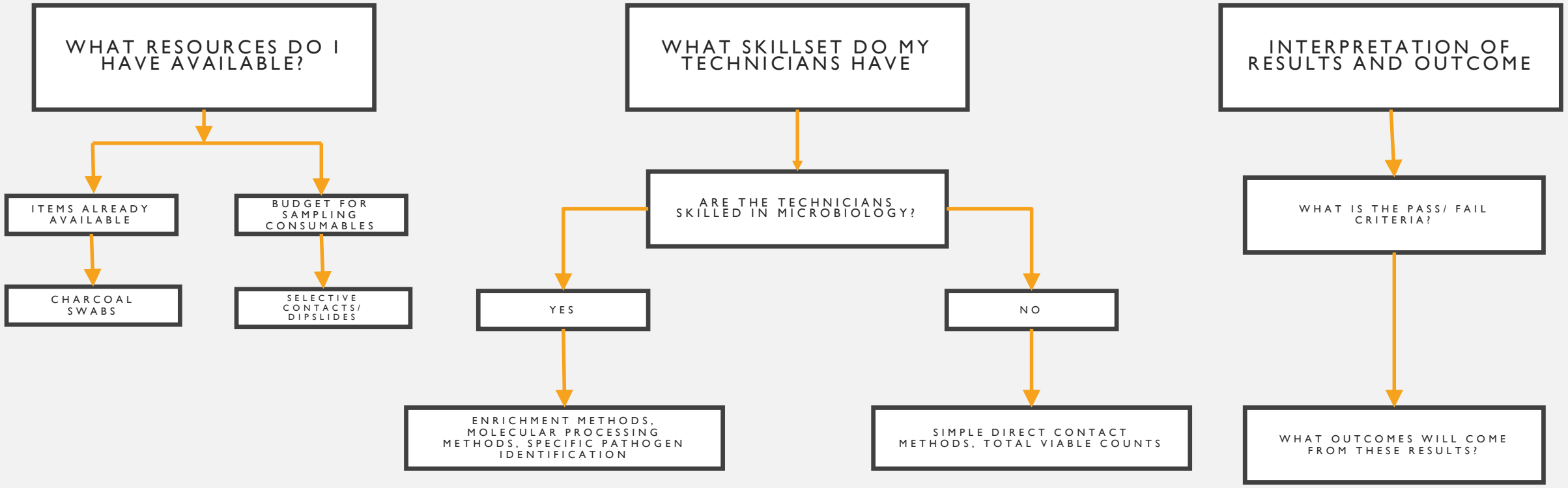
CONS

- Possibility of contamination unless excellent aseptic technique is used.
- Post-test processing losses.
- Difficult to reproduce methodology.

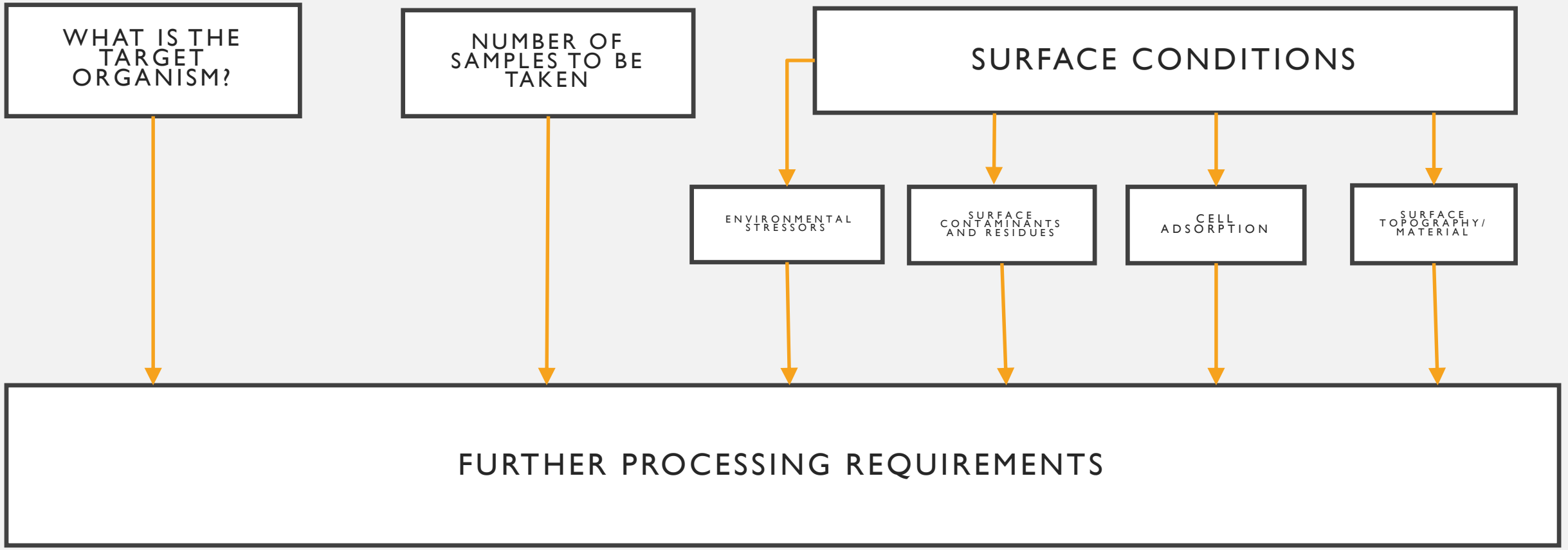
WHAT IS MY CONCERN?



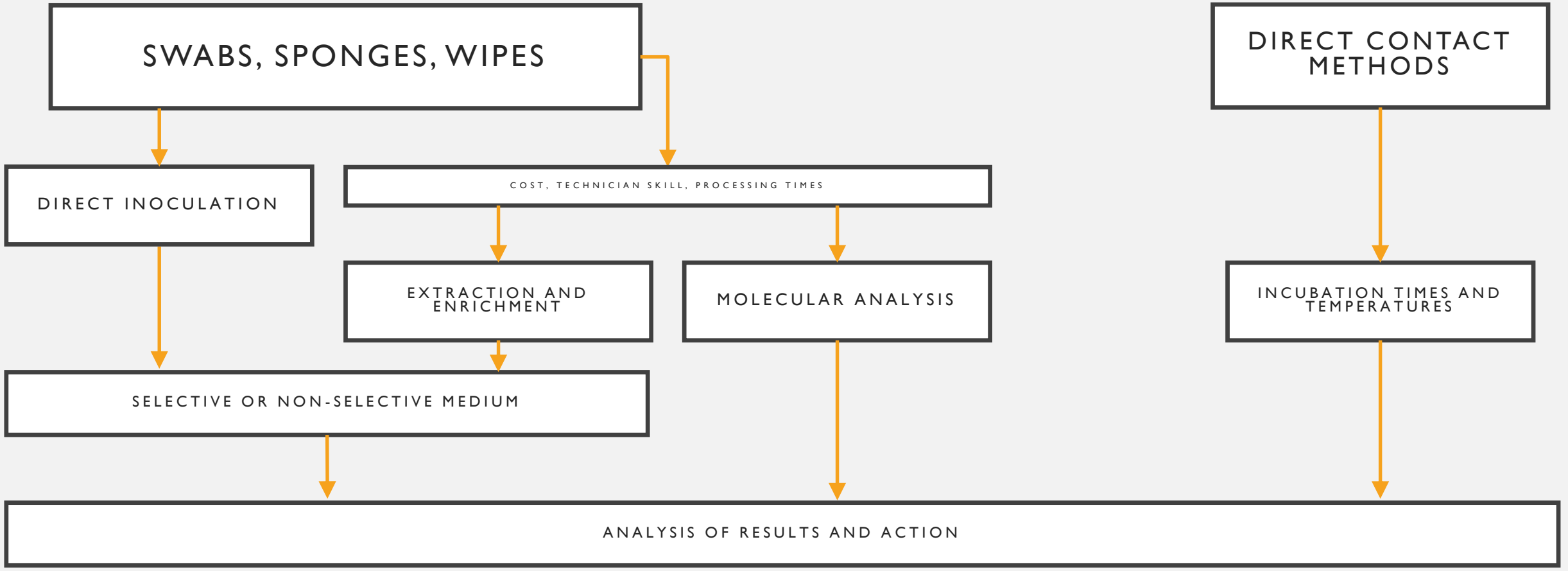
CONSIDERING SAMPLING METHODS



CHOOSING A SAMPLING METHOD



POST-TEST PROCESSING



CONSIDERATIONS OF MY LITERATURE REVIEW

CONTAMINATION OF HOSPITAL SURFACES

- MDRO's are being recovered in the near-patient environment.
- Cleaning and terminal cleaning is insufficient as pathogens are still being isolated, which poses a risk to future occupants.
- Patients shed into their environment.
- High-touch surfaces nearest to the patient are the most contaminated.
- Reported surface bioburden varies greatly between all studies.

HOW TO SAMPLE SURFACES

- Sampling competency is more important than optimum method selection.
- There is no legislation to enforce environmental monitoring of surfaces in the hospital environment.
- Hospitals are choosing swabs most frequently for sampling.
- Different conditions call for different sampling methods; no single sampling method is suitable for all conditions.
- Sponges are the best method for sampling *C. difficile.*
- Swabs with enrichment are the best method for sampling MRSA.
- Direct contact methods work better for adsorbed cells.
- Dipslides should be investigated further as they show great promise as a sampling method.
- Most sampling losses occur during processing; method selection plays a lesser role.
- Results between studies are incomparable, incomplete and contradictory. Further investigations are needed under varying experimental conditions are needed to improve surface sampling methodologies.
- Differences in recoveries between methods is reported, though often to no statistical significance.

EU GUIDANCE 'THE ORANGE GUIDE'

- Guidance on Good Manufacturing Practise (Annex 1).
- Guidance document *Manufacture of Sterile products.*
- Guidance on when/how to use sampling methods (settle plates, contact plates, swabs, finger dabs, air sampling) to monitor cleanrooms.
- Overall allowed contamination levels given, broken down by grade and method of sampling.

	Recommended limits for microbial contamination			
Grade	Air sample cfu/m3	Settle plates cfu/4 hrs	Contact Plates cfu/plate	Gloves print cfu/ glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Table produced from recommended limits for monitoring clean areas during operation. EU Guidelines to Good Manufacturing Practise Medicinal Products for Human and Vetinarty Use. Annex 1.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

ISO 18593:2004; MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS - HORIZONTAL METHODS FOR SAMPLING TECHNIQUES FROM SURFACES USING CONTACT PLATES AND SWABS

→ METHODS FOR SAMPLING TECHNIQUES USING CONTACT PLATES OR SWABS ON SURFACES IN THE FOOD INDUSTRY AND FOOD PROCESSING PLANTS; A VIEW OF DETECTING OR ENUMERATING VIABLE MICROORGANISMS.

ISO 14644; TESTING AND MONITORING CLEANROOMS OR CLEAN AREAS, INCLUDING SURFACE PARTICLE CLEANLINESS

→ HOW OFTEN TO UNDERTAKE PARTICLE COUNTING, EQUIPMENT METHODOLOGY, GUIDE ON FINGER DAB TESTING, MEDIA CHOICES FOR DIFFERENT TESTING CIRCUMSTANCES, PROPOSED ACTION AND WARNING LIMITS.

ISO Class	Work surfaces CFU/ plate	Non-work surfaces CFU/ plate
5	>3	N/A
7	>5	>10
8	>25	>50

UNITED STATES PHARMACOPEIA <1116>

- Microbiological evaluation of cleanrooms and controlled environments.
- Suggested limits and information on investigations following exceeding of microbiological limits.
- How to implement a good environmental monitoring programme.
- Establishing a sampling plan and where to sample, and frequency.
- Culture media and diluents to use for microorganism recovery and quantification, incubation conditions, times and temperatures.
- USP Chapter 1113 - Microbial Characterization, Identification, and Strain Typing, workflow for microbial identification.

Room Classification	Active air sample (%)	Settle Plate (9cm) 4hr exposure (%)	Contact plate/ swab (%)	Glove/ garment (%)
Isolator/ISO 5+	<0.1	<0.1	<0.1	<0.1
ISO 5	<1	<1	<1	<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

Suggested initial contamination recovery rate (rate at which any contamination is found, the incidence) in aseptic environments, adapted from Table 3 USP <1116>

FDA

- (US Food and Drug Administration) aseptic processing guidance document.
- Details on acceptable environmental monitoring methods.
- Where to sample (product contact surfaces, floors, walls, equipment).
- Guidance on environmental trending.

Classification (0.5 um particles/ft3)	ISO	> o.5 um particles/m3	Microbiological active air action levels (cfu/m3)	Settle plates action level 90mm cfu/4 hrs
100	5	3,520	<1	<1
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

Recommended microbiological action limits for clean rooms adapted from FDA pharmaceutical guidelines

SUGGESTED READING

RECOVERY OF ORGANISMS FROM HOSPITAL ENVIRONMENTS

- Lemmen, S. W., H. Hafner, D. Zollman, S. Stanzel and R. Lutticken (2004). "Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment." Journal of Hospital Infection **56**(3): 191-197.
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- Gbaguidi-Haore, H., D. Talon, D. Hocquet and X. Bertrand (2013). "Hospital environmental contamination with Enterobacteriaceae producing extended-spectrum beta-lactamase." American Journal of Infection Control **41**(7): 664-665.

SAMPLING METHODS

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- Dolan, A., M. Bartlett, B. McEntee, E. Creamer and H. Humphreys (2011). "Evaluation of different methods to recover meticillin-resistant Staphylococcus aureus from hospital environmental surfaces." Journal of Hospital Infection **79**(3): 227-230.

THANK YOU