MICROCHEM LABORATORY

STUDY REPORT

Study Title

Determination of Environmental Microorganisms in Hyve's Swab Samples

Test Method

Environmental Swab Study

Study Identification Number

NG15285

Study Sponsor

Hyve
info@hyveclean.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

Environmental Swab Analysis: General Information

Surveillance of environmental surfaces using swab analysis is a common microbiological technique to monitor the microbial population on various environmental sites. The assay provides quantified results of the number of heterotrophic (carbon-utilizing) bacteria at each site that was sampled using a sampling sponge or stick transported in a neutralizing medium. A variety of specialized plating media types may also be used to selectively and differentially quantify microorganisms of interest. Results from environmental swab analysis studies may be used to ensure appropriate disinfection procedures, monitor cleanroom surfaces, and gauge the success of treated surfaces in the field.

Laboratory Qualifications Specific to Environmental Swab Studies

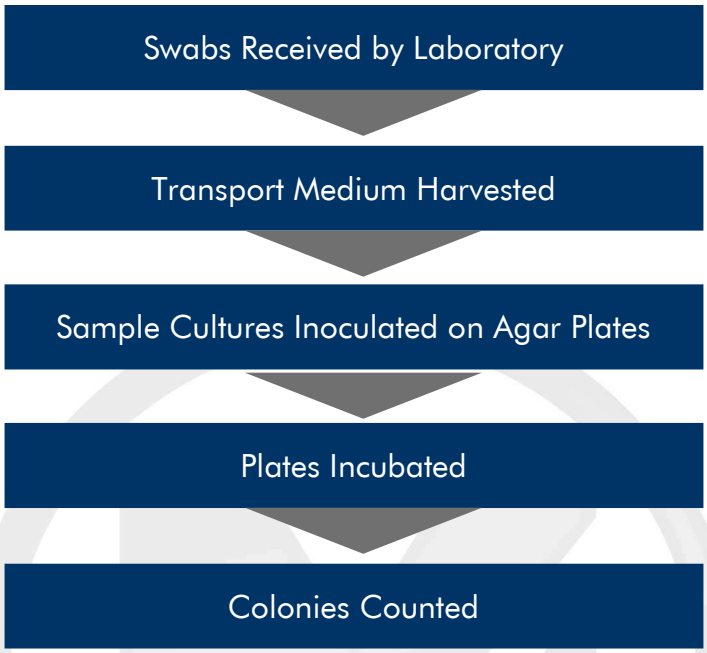
Microchem Laboratory began conducting environmental swab studies in 2008. Since then, the laboratory has gained a great deal of experience via numerous swab studies on a broad array of environmental surfaces. Microchem scientists have performed on-site sampling at medical facilities, cleanrooms, and other locations of interest, and processed swab samples taken by Study Sponsors. Swabs have been used to quantify general microbiological contamination levels or specific species of interest.

Study Timeline

Swabs Received	Swabs Plated	Plates Incubated	Plates Harvested	Plates Evaluated	Report Delivered
1: 27 APR 2020	28 APR 2020	28 APR 2020	30 APR 2020	30 APR 2020	
2: 29 APR 2020	29 APR 2019	29 APR 2019	01 MAY 2020	01 MAY 2020	08 MAY 2020
3: 01 MAY 2020	01 MAY 2020	01 MAY 2020	03 MAY 2020	04 MAY 2020	

1: Baseline, 2 hour, 5 hour, 12 hour, and 24 hour swabs received and plated
2: 72 hour swabs received and plated
3: 120 hour swabs received and plated

Diagram of the Procedure



Summary of the Procedure

- Environmental surfaces are sampled using sterile swabbing supplies (may be provided by Microchem Laboratory) and good aseptic technique.
- Swab samples are recovered in a minimally nutritive recovery medium and shipped under refrigerated conditions to Microchem Laboratory.
- Upon receipt, the condition of received swab samples is observed by the laboratory.
- Swabs samples are serially diluted, if appropriate, and dilutions are plated to the appropriate growth-supporting plating medium.
- Enumeration plates are incubated under the appropriate growth conditions for the target microorganisms.
- The number of microorganisms recovered from each sample is observed following the incubation period.

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Criteria for Scientific Defensibility of an Environmental Swab Study

For Microchem Laboratory to consider an environmental swab study to be scientifically defensible, the following criteria must be met:

1. All swab, dilution, and plating media used in the study must be sterile.
2. Selective agar used must demonstrate appropriate growth of control microorganisms.

Passing Criteria

Passing criteria for this assay is not specified, however, the passing criteria may be determined by the Study Sponsor.

Testing Parameters used in this Study

Sample Type:	Sponge Swab	**Number of Samples:**	14
Transport Medium:	Neutralizing Buffer	**Culture Dilution Media:**	Phophate Buffered Saline
Enumeration Plate Media:	Tryptic Soy Agar		
	HardyCHROM ECC Agar (*E. coli*)	**Enumeration Plate Incubation Temperature:**	36°C ± 2°C
	HardyCHROM Salmonella Agar		
		Enumeration Plate Incubation Time:	2 days

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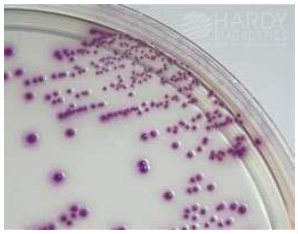
Study Modifications

No further modifications were made to the method for this study.

Study Notes

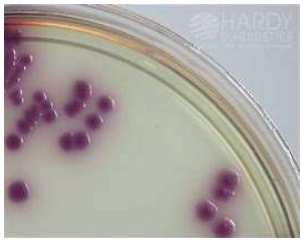
No additional observations or notations were made for this study.

Targeted Organisms of Interest



Escherichia coli

This bacterium is a Gram-negative, rod-shaped, facultative anaerobe. *E. coli* is typically a harmless microbe that naturally occupies the gastrointestinal tract, but some strains demonstrate pathogenicity for urinary tract infections, neonatal meningitis, and gastroenteritis. *E. coli* is commonly used in several test methods as a model for gram negative bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.



Salmonella enterica

This bacterium is a Gram-negative, rod-shaped, facultative aerobe. *S. enterica* is found commonly in the intestinal tract of mammals, birds, and reptiles and typically transmitted through the consumption of foods of animal origin. *S. enterica* is a pathogen for salmonellosis, a disease that can lead to fever, abdominal pain, vomiting, diarrhea, and in some cases, can be life-threatening *Salmonella* spp. remain one of the most common foodborne pathogens in the world today, and the genus' resistance to antimicrobials and disinfectants have notably increased over the years.

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Control Results

Growth Confirmation: Yes

Media Sterility: Yes

Calculations

$$CFU/mL = \frac{(Count1 + Count2)}{2} * 10^{DF}$$

Where:

- CFU is Colony Forming Units
- DF is the Dilution Factor plated

CFU/Sample = CFU/mL * Total Sample Volume

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Results of the Study

Sample	Sample ID	HardyCHROM Salmonella CFU/Sample	HardyCHROM ECC (*E. coli*) CFU/Sample
Hyve	Baseline	0.00E+00	0.00E+00
	2 hr	0.00E+00	0.00E+00
	5 hr	0.00E+00	0.00E+00
	12 hr	0.00E+00	0.00E+00
	24 hr	0.00E+00	0.00E+00
	72 hr	0.00E+00	0.00E+00
	120 hr	0.00E+00	0.00E+00
Competitor	Baseline	0.00E+00	0.00E+00
	2 hr	0.00E+00	0.00E+00
	5 hr	0.00E+00	0.00E+00
	12 hr	0.00E+00	0.00E+00
	24 hr	0.00E+00	0.00E+00
	72 hr	5.00E+01	0.00E+00
	120 hr	2.20E+04	0.00E+00

Findings of the Study

With the introduction of Salmonella at 72 hours, the Hyve product was able to eliminate it via bio-control of the probiotics in the Hyve product. On the competitor's side, the salmonella was able to flourish without much competition.

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.