HYcheck™

HYcheck D/E Neutralizing Agar · HYcheck for Disinfection Control · HYcheck for Enterobacteriaceae · HYcheck Plate Count Agar With TTC · HYcheck for Total Count · HYcheck for Yeasts and Molds · HYcheck for Yeasts and Molds with TTC

Intended Use

HYcheck is a hygiene contact slide which is used for assessing the microbiological contamination of surfaces or fluids.

Summary and Explanation

Monitoring the microbial flora of environmental surfaces, walls, ceilings, and equipment is an important stage in achieving good manufacturing practices in factories handling foods, cosmetics or pharmaceuticals.[1,2,3] To maintain good hygiene standards in hotels and restaurant kitchens, microbiological contamination must also be monitored.[4] Methods to monitor the environmental flora have been described using either swabbing techniques[5] or contact plates.[6] Contact slides were created to monitor the microbial flora of liquids (e.g. urine, milk) and equipment surfaces in the clinical and food industries.[1] Contact slides are statistically comparable to swab and contact plates for surface sampling.[1] The HYcheck contact slides were developed for the testing of fluids and surfaces for microbial cleanliness.

HYcheck is a double sided, hinged plastic paddle containing two agar surfaces. The agar surface extends above the paddle allowing for contact with test surfaces. The hinged paddle allows the agar surface to be easily held against each test area during sampling. The surface area of the paddle is clearly divided into seven units of one centimeter each to allow direct counting of microbial density per unit area.

The HYcheck range of hygiene control slides consists of seven media combinations designed to meet the various needs for monitoring different types of microbial contamination.

HYcheck D/E Neutralizing Agar has both sides coated with D/E Neutralizing Agar, a medium developed by Dey and Engley[7] to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. D/E Neutralizing Agar neutralizes higher concentrations of residual antimicrobials, when compared with other standard neutralizing formulas, such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[8,9] Complete neutralization of disinfectants is important because disinfectant carryover can result in a false no growth result. D/E Neutralizing media effectively neutralize the inhibitory effects of disinfectant carryover,[10,11] allowing differentiation between bacteriostasis and true bactericidal actions of disinfectant chemicals.

User Quality Control

Identity Specifications

HYcheck D/E Neutralizing Agar

Medium:	**D/E Neutralizing Agar**
Appearance:	Lavender
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.6 ± 0.2

HYcheck for Disinfection Control

Media:	**D/E Neutralizing Agar**	**Tryptic Soy Agar**
Appearance:	Lavender	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.6 ± 0.2	7.3 ± 0.2

continued on following page

HYcheck for Enterobacteriaceae

Media:	**Violet Red Bile Glucose Agar**	**Tryptic Soy Agar**
Appearance:	Reddish purple	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.4 ± 0.2	7.3 ± 0.2

HYcheck Plate Count Agar with TTC

Medium:	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.0 ± 0.2

HYcheck for Total Count

Media:	**Plate Count Agar**	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.0 ± 0.2	7.0 ± 0.2

HYcheck for Yeasts and Molds

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

HYcheck for Yeast and Molds with TTC

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar with 0.01% TTC**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

Cultural Response (approx inoculum 30-300 CFU)

HYcheck D/E Neutralizing Agar

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E AGAR
Aspergillus niger NCPF 2275	–	good
Bacillus subtilis	6633	good
Candida albicans	2091	good
Escherichia coli	25922*	good
Pseudomonas aeruginosa	27853*	good
Staphylococcus aureus	25923*	good
Staphylococcus epidermidis	12228*	good

HYcheck for Disinfection Control

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Bacillus subtilis	6633	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	good	good
Pseudomonas /nosa	27853*	good	good
Staphylococcus aureus	25923*	good	good
Staphylococcus epidermidis	12228*	good	good

HYcheck for Enterobacteriaceae

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON VRBGA	GROWTH ON TSA
Enterobacter aerogenes	13048*	good	good
Enterococcus faecalis	19433*	none to poor	good
Escherichia coli	25922*	good	good
Proteus mirabilis NCTC 11938	–	good	good
Salmonella typhimurium	14028*	good	good
Shigella sonnei	25931*	good	good
Staphylococcus aureus	25923*	none to poor	good

HYcheck Plate Count Agar with TTC

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good
Escherichia coli	25922*	good
Proteus vulgaris	13315	good
Salmonella typhimurium	14028*	good
Staphylococcus aureus	25923*	poor

HYcheck for Total Count

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good	good
Escherichia coli	25922*	good	good
Proteus vulgaris	13315	good	good
Salmonella typhimurium	14028*	good	good
Staphylococcus aureus	25923*	good	poor

HYcheck for Yeasts and Molds

Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	good
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	good
Streptococcus pyogenes	19615*	none to poor	good

HYcheck for Yeasts and Molds with TTC

Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA W/TTC
Aspergillus niger NCPF 2275	—	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	poor
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	poor
Streptococcus pyogenes	19615*	none to poor	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

HYcheck for Disinfection Control has side one coated with D/E Neutralizing Agar (D/E) (see above), and side two coated with Tryptic Soy Agar (TSA). In 1955, Leavitt et al.[12] demonstrated that Tryptic Soy Agar supports excellent growth of a both aerobic and anaerobic microorganisms. Tryptic Soy Agar is a general purpose medium that is recommended in multiple water and wastewater applications.[13]

HYcheck for Enterobacteriaceae has side one coated with Violet Red Bile Glucose Agar and side two coated with Tryptic Soy Agar, a general purpose growth medium. Violet Red Bile Glucose Agar is a selective medium used for the enumeration of *Enterobacteriaceae* in foods. Coliform bacteria have long been used as an index of fecal contamination in waters, and their presence in milk is used as an index of sanitation in milk processing.[14] The presence of *Enterobacteriaceae*, coliforms, *Salmonellae*, *Klebsiella* or *Citrobacter*, in raw foodstuffs is an indicator of fecal contamination. Their presence after processing may indicate a failure in the manufacturing process.

HYcheck Plate Count Agar with TTC has both sides coated with Plate Count Agar with TTC (0.01% 2,3,5-Triphenyl Tetrazolium Chloride).

HYcheck for Total Count has side one coated with Plate Count Agar and side two coated with Plate Count Agar with 0.01% TTC. Plate Count Agar is used for enumerating bacteria in water, wastewater, food and dairy products.[13,15-18] TTC is a redox indicator that is colorless in the oxidized form. TTC is reduced to insoluble triphenylformazan by certain actively metabolizing bacteria, resulting in a red color in the presence of bacterial growth.

There are two HYcheck products for yeasts and molds: 1) **HYcheck for Yeasts and Molds** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar; 2) **HYcheck for Yeasts and Molds with TTC** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar with 0.01% TTC. Rose Bengal Chloramphenical Agar is recommended in the selective isolation and enumeration of yeasts and molds from environmental materials and foodstuffs. The pH of the medium is near neutrality for improved growth and recovery of acid sensitive strains. [19-21]

Principles of the Procedure

HYcheck D/E Neutralizing Agar

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

HYcheck for Disinfection Control

D/E Neutralizing Agar (D/E) - side one

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

Tryptic Soy Agar (TSA) - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck for Enterobacteriaceae

Violet Red Bile Glucose Agar (VRBGA) - side one

Yeast Extract provides vitamins, cofactors, nitrogen and carbon. Glucose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck Plate Count Agar (PCA) with TTC

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Total Count

Plate Count Agar - side one

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Plate Count Agar with TTC - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds

Rose Bengal Chloramphenicol Agar (RBCA) - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal and Chloramphenicol inhibit bacterial growth and restrict size and height of rapidly growing mold colonies. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds with TTC

Rose Bengal Chloramphenicol Agar - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal suppresses bacterial growth and restricts size and height of rapidly growing mold colonies. Chloramphenicol inhibits bacteria. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

Precautions

1. Do not touch agar surface.
2. Do not use if there are signs of dehydration or contamination.

Storage

Store HYcheck slides at 2-15°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

(One type is provided per package.)

HYcheck D/E Neutralizing Agar
HYcheck for Disinfection Control
HYcheck for Enterobacteriaceae
HYcheck Plate Count Agar with TTC
HYcheck for Total Count
HYcheck for Yeasts and Molds
HYcheck for Yeasts and Molds with TTC.

Test Procedure

Surfaces

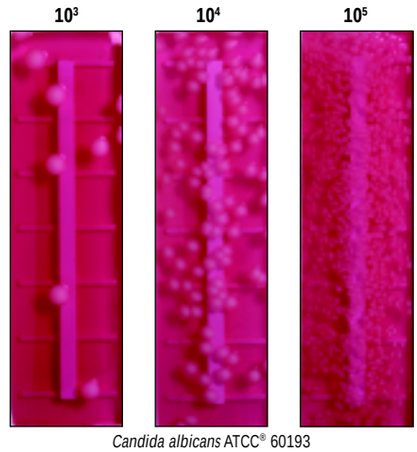
1. Loosen cap and remove HYcheck slide from the container.
2. Examine for dehydration or contamination.
3. Hold terminal spike against surface to be tested.
4. Press down on the spike to bend the paddle around the hinge line.
5. Gently lower the slide and press agar into contact with the test surface.
6. Apply firm and even pressure on the test surface for a few seconds.
7. Repeat procedure using the second agar surface on an area adjacent to the initial test site.
8. Replace slide in the container and close tightly.
9. Incubate in an upright position at indicated temperature.

Liquids

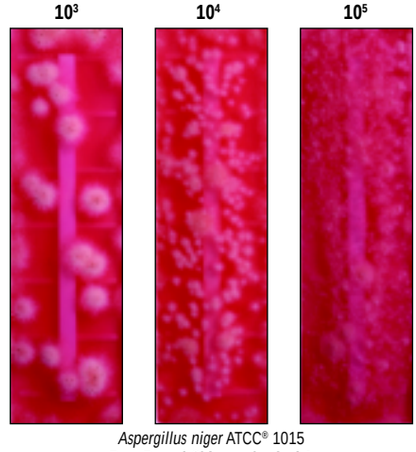
1. Loosen cap and remove HYcheck Slide from the container.
2. Examine for dehydration or contamination.
3. Immerse slide into test fluid so that agar surface becomes totally covered (if insufficient liquid is available, pour over surface of the slide).
4. Allow to drain.
5. Replace slide in the container and close tightly.
6. Incubate in an upright position at indicated temperature.

Results

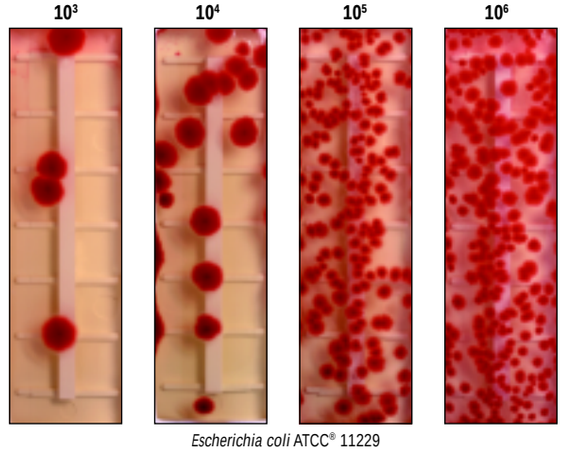
The following photos are reactions to *Candida albicans, Aspergillus niger and Escherichia coli.*



Candida albicans ATCC® 60193
on Rose Bengal Chloramphenicol Agar



Aspergillus niger ATCC® 1015
on Rose Bengal Chloramphenicol Agar



Escherichia coli ATCC® 11229
on Tryptic Soy Agar with 0.01% TTC

Limitations of the Procedure

1. Do not use the HYcheck Slide if it is contaminated or the agar medium is significantly dehydrated.

References

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Packaging

HYcheck D/E Neutralizing Agar	20 units	9041-36
HYcheck for Disinfection Control	20 units	9039-36
HYcheck for Enterobacteriaceae	20 units	9037-36
HYcheck Plate Count Agar with TTC	20 units	9045-36
HYcheck for Total Count	20 units	9053-36
HYcheck for Yeasts and Molds	20 units	9038-36
HYcheck for Yeasts and Molds with TTC	20 units	9046-36