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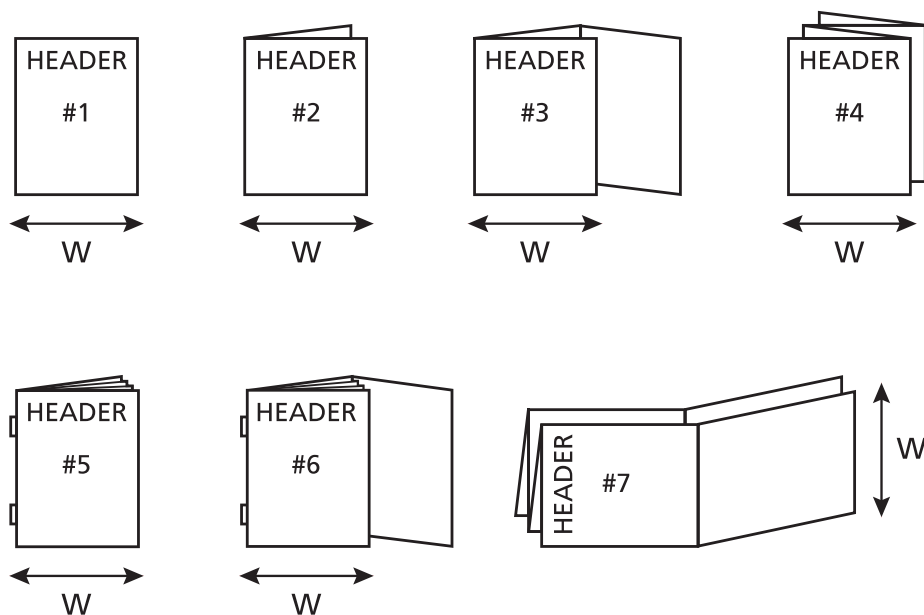
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**Becton, Dickinson and Company**  
7 Loveton Circle  
Sparks, MD 21152 USA

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By Sonia F.Thompson at 1:48 pm, May 22, 2018

**Proofing Approved By:** **PROOFING APPROVED BY**  
By Sarah Henderson at 1:49 pm, May 22, 2018

**Third Eye By:** **THIRD EYE BY**  
By Maria Zacharias at 1:12 pm, Jun 01, 2018

The hygiene contact slide with the unique hinge for monitoring microbial contamination on surfaces or in liquids.

L005259(02)  
2018-05  
English

# Change History

Revision	Pages/Sections	Change Summary
02	All	Update BD Branding



## The hygiene contact slide with the unique hinge for monitoring microbial contamination on surfaces or in liquids.

L005259(02)  
2018-05  
English

### INTENDED USE

**BD Difco™ Hycheck™** is a hygiene contact slide used to assess 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.<sup>1-3</sup> To maintain good hygiene standards in hotels and restaurant kitchens, microbiological contamination must also be monitored.<sup>4</sup> Methods to monitor the environmental flora have been described using either swabbing techniques<sup>5</sup> or contact plates.<sup>6</sup> Contact slides were created to monitor the microbial flora of liquids (e.g. urine, milk) and equipment surfaces in the clinical and food industries.<sup>1</sup> Contact slides are statistically comparable to swab and contact plates for surface sampling.<sup>1</sup> The **BD Difco Hycheck** contact slides were developed for the testing of fluids and surfaces for microbial cleanliness.

**BD Difco 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 **BD Difco Hycheck** range of hygiene contact slides consists of seven media combinations designed to meet the various needs for monitoring different types of microbial contamination.

### PRINCIPLES OF THE PROCEDURE

Pancreatic digest of casein and other peptones provide carbon and nitrogen. Yeast extract provides vitamins, cofactors, nitrogen and carbon. Papaic digest of soybean meal provides nitrogen, vitamins and minerals. Dextrose (glucose) provides fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

In **BD Difco D/E Neutralizing Agar**, sodium thioglycollate neutralizes mercurial compounds. Sodium thiosulfate neutralizes iodine and chlorine. Sodium bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Bromocresol purple is a colorimetric indicator.

In **BD BBL Violet Red Bile Glucose Agar**, bile salts and crystal violet inhibit gram-positive bacteria. Glucose fermenters produce red colonies with red-purple halos in the presence of neutral red, a pH indicator.

In **BD BBL Rose Bengal Chloramphenicol Agar**, monopotassium phosphate provides buffering capacity. Magnesium sulfate provides necessary trace elements. Rose bengal is a selective agent that inhibits bacterial growth and restricts the size and height of bacterial colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Chloramphenicol inhibits bacteria.

In **BD Difco Plate Count Agar with TTC**, triphenyl tetrazolium chloride (TTC) is a redox indicator.

### REAGENTS

#### **BD Difco D/E Neutralizing Agar**

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	5.0 g
Yeast Extract .....	2.5 g
Dextrose .....	10.0 g
Sodium Thioglycollate .....	1.0 g
Sodium Thiosulfate .....	1.0 g
Sodium Bisulfite .....	2.5 g
Lecithin .....	7.0 g
Polysorbate 80 .....	5.0 g
Bromocresol Purple .....	0.02 g
Agar .....	15.0 g

#### **BD Difco Tryptic Soy Agar**

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	15.0 g
Papaic Digest of Soybean Meal .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	15.0 g

### BD BBL Violet Red Bile Glucose Agar

Approximate Formula\* Per Liter Purified Water

Yeast Extract .....	3.0 g
Peptone .....	7.0 g
Dextrose .....	10.0 g
Sodium Chloride .....	5.0 g
Bile Salts No. 3 .....	1.5 g
Neutral Red .....	0.03 g
Crystal Violet .....	0.002 g
Agar .....	15.0 g

### BD Difco Plate Count Agar (PCA)

Approximate Formula\* Per Liter Purified Water

Pancreatic Digest of Casein .....	5.0 g
Yeast Extract .....	2.5 g
Dextrose .....	1.0 g
Agar .....	15.0 g

**BD Difco Plate Count Agar (PCA) with TTC** contains 0.1 g/L in addition to the above ingredients.

## PROCEDURE

**Materials Provided:** One type is provided per package of the following: **BD Difco Hycheck D/E Neutralizing Agar**, **BD Difco Hycheck Disinfection Control**, **BD Difco Hycheck Enterobacteriaceae**, **BD Difco Hycheck Plate Count Agar with TTC**, **BD Difco Hycheck Total Count**, **BD Difco Hycheck Yeasts and Molds** and **BD Difco Hycheck Yeasts and Molds with TTC**.

## BD DIFCO HYCHECK: THE COMPLETE RANGE

The **BD Difco Hycheck** range of hygiene contact slides consists of seven products designed to meet the various needs for monitoring different types of microbial contamination. All the slides contain two agar surfaces.

Most products have one agar to give a non-selective total count. The other agar surface is designed to identify or select a specified group of microorganisms. The exceptions to this are **BD Difco Plate Count Agar with TTC** and **BD Difco D/E Neutralizing products** which have the same medium on both sides, and the **Total Count** product which has two non-selective media.

## METHOD OF USE: GENERAL

### 1 REMOVE SLIDE

Loosen cap on the tube and remove the **BD Difco Hycheck** Slide from the container taking care not to touch the agar surface. Examine for any dehydration or contamination.



### 2 SAMPLING

#### a. Surfaces

Hold the terminal spike against the surface to be tested. Press down on the spike to bend the paddle around the hinge line. Gently lower the slide and press the agar into contact with the test surface, still holding the slide by the cap.



Apply a firm and even pressure on the test surface for a few seconds taking care not to smear the agar over the test area.

### BD BBL Rose Bengal Chloramphenicol Agar (RBCA)

Approximate Formula\* Per Liter Purified Water

Papaic Digest of Soybean Meal .....	5.0 g
Dextrose .....	10.0 g
Monopotassium Phosphate .....	1.0 g
Magnesium Sulfate .....	0.5 g
Rose Bengal .....	0.05 g
Agar .....	15.0 g
Chloramphenicol .....	0.05 g

\*Adjusted and/or supplemented as required to meet performance criteria.

**Precautions:** For Laboratory Use

## STORAGE

Store **BD Difco Hycheck** at 2–8 °C with the arrow on the carton label pointing up in a vertical position.

**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.

Repeat the procedure using the second agar surface on an area adjacent to the initial test site. Replace slide in the container and close tightly.

#### b. Liquids

Immerse the **BD Difco Hycheck** Slide into the test fluid so that the agar surface becomes totally covered. If insufficient liquid is available, pour over the surface of the slide. Allow to drain. Replace the slide in the container and close tightly.

**3 LABEL** – Enter the site, location, date and time. Also indicate whether the sample has been taken before or after cleaning.

**4 INCUBATION** – Incubate in an upright position at the temperature indicated in the instructions for the particular product. However, alternative times and temperatures for incubation may be used according to individual requirements.

**5 READING RESULTS** – Remove slide from the tube and count the number of colonies on each agar surface. Reflected light facilitates reading.

### Enterobacteriaceae

**Side 1** - Coated with **BD BBL Violet Red Bile Glucose Agar**, a clear red medium used for the enumeration of *Enterobacteriaceae* in foods. The presence of *Enterobacteriaceae*, coliforms, *Salmonellae*, *Klebsiella* or *Citrobacter* spp. in raw foodstuffs is an indicator of faecal contamination. Their presence after processing may indicate a failure in the manufacturing procedure.

**Side 2** - Coated with **BD Difco Tryptic Soy Agar**, this clear medium will give a total aerobic bacterial count of the test surface.

### INCUBATION

Incubate in an upright position at 35–37 °C for 24 h. Examine the slide again after a further 24 h incubation. Other times and temperature of incubation may be used dependent upon the requirements of the environment tested.

### READING RESULTS

**BD Difco Tryptic Soy Agar:** Count the total number of colonies on the slide after 48 h incubation to obtain the total bacterial count of the test surface.

**BD BBL Violet Red Bile Glucose Agar:** *Enterobacteriaceae* will ferment the glucose in the medium to produce acid. The resulting drop in pH of the medium causes bile salts to precipitate around the red colonies as a red opaque halo. Non-glucose fermenters remain colorless on the medium and show no bile halo. Most gram-positive organisms are inhibited on the medium. Count the red colonies surrounded by a precipitate of bile to obtain the *Enterobacteriaceae* count.

### TYPICAL CULTURAL RESPONSE

Organism	BD Difco Tryptic Soy Agar (24 hours)	V.R.B.G.A. (24 hours)
* <i>Klebsiella aerogenes</i> ATCC® 13048	Good growth	Good growth
* <i>Escherichia coli</i> ATCC 25922	Good growth of cream moist colonies	Good growth of red colonies surrounded by an opaque red halo
<i>Proteus mirabilis</i> NCTC™ 11938	Good growth	Good growth
* <i>Shigella sonnei</i> ATCC 25931	Good growth	Good growth
* <i>Salmonella typhimurium</i> ATCC 14028	Good growth of cream moist colonies	Good growth of red colonies surrounded by an opaque red halo
* <i>Staphylococcus aureus</i> ATCC 25923	Good growth of cream/yellow colonies	Growth inhibited
* <i>Enterococcus faecalis</i> ATCC 19433	Good growth of small white colonies	Growth inhibited

\*Cultures listed above may be available as Microtrol™ (UK) or Bactrol™ Plus (USA) Disks.

### BD Difco Hycheck - Yeasts and Molds

**Side 1** - Coated with **BD BBL Rose Bengal Chloramphenicol Agar**, a pink agar recommended for the selective isolation of yeasts and molds from environmental materials and foodstuffs. The pH of the medium is near neutral for improved growth of acid-sensitive strains.

**Side 2** - Coated with **BD Difco Tryptic Soy Agar**, a clear colorless medium recommended in the *European Pharmacopoeia* and *The United States Pharmacopoeia* for microbial limits testing. The medium will give a total aerobic count.

### INCUBATION

Incubate in an upright position at 30 °C. Record the count on the **BD Difco Tryptic Soy Agar** after 48 h incubation and then on the **BD BBL Rose Bengal Chloramphenicol Agar** after 120 h incubation. Other times and temperature of incubation may be used dependent upon the requirements of the environment tested.

### READING RESULTS

**BD Difco Tryptic Soy Agar:** Record the total number of colonies on the **BD Difco Tryptic Soy Agar** to obtain the total aerobic bacterial count.

**BD BBL Rose Bengal Chloramphenicol Agar:** Count the total number of colonies on the agar to obtain an assessment of the fungal contamination (colonies of yeasts appear pink due to uptake of **BD BBL Rose Bengal**). Bacterial growth is inhibited on this medium.

#### TYPICAL CULTURAL RESPONSE

Organism	BD Difco Tryptic Soy Agar (48 hours)	BD BBL Rose Bengal Chloramphenicol Agar (5 days)
<i>Candida albicans</i> ATCC 2091	Good growth of white raised colonies	Good growth of pink smooth colonies
<i>Aspergillus niger</i> NCPF™ 2275	Good growth appearing white and filamentous and becoming black after 5 days incubation	Good growth appearing white and filamentous and becoming black after 5 days incubation
<i>Saccharomyces cerevisiae</i> NCYC™ 1211	Good growth	Good growth
* <i>Serratia marcescens</i> ATCC 8100	Good growth	Inhibited
* <i>Escherichia coli</i> ATCC 25922	Good growth of cream moist colonies	Growth restricted
* <i>Staphylococcus aureus</i> ATCC 25923	Good growth of cream/yellow colonies	Growth restricted
* <i>Streptococcus pyogenes</i> ATCC 19615	Good growth	Inhibited

\*Cultures listed above may be available as Microtrol (UK) or **Bactrol** Plus (USA) Disks.

#### BD Difco Hycheck - Yeasts and Molds with TTC

This product is the same as the **BD Difco Hycheck** Yeast and Molds product except that 0.01% triphenyl tetrazolium chloride (TTC) is incorporated into the **BD Difco** Tryptic Soy Agar. In an oxidized state TTC is colorless but on reduction by microorganism growth an insoluble red formazan dye is produced.

This aids counting of normally colorless or translucent colonies. The product should be used as described for the standard Yeasts and Molds **BD Difco Hycheck** product. Some organisms, e.g. staphylococci, may grow less profusely on the medium containing TTC and some yeasts, e.g. *Saccharomyces cerevisiae*, are inhibited completely.

#### TYPICAL CULTURAL RESPONSE

Organism	BD Difco Tryptic Soy Agar Plus TTC (48 hours)	BD BBL Rose Bengal Chloramphenicol Agar (5 days)
<i>Aspergillus niger</i> NCPF 2275	Good growth	Good growth
* <i>Candida albicans</i> ATCC 2091	Good growth, red dye reduction	Good growth of pink smooth colonies
<i>Saccharomyces cerevisiae</i> NCYC 1211	Growth inhibited	Good growth of pink colonies
* <i>Escherichia coli</i> ATCC 25922	Good growth, red dye reduction	Growth restricted
* <i>Serratia marcescens</i> ATCC 8100	Good growth	Inhibited
* <i>Staphylococcus aureus</i> ATCC 25923	Trace growth, red dye reduction	Growth restricted
* <i>Streptococcus pyogenes</i> ATCC 19615	Good growth	Inhibited

\*Cultures listed above may be available as Microtrol (UK) or **Bactrol** Plus (USA) Disks.

#### BD Difco Hycheck - Disinfection Control

**Side 1** - Coated with **BD Difco** D/E Neutralizing Agar, a purple agar gel used for determining the efficiency of sanitization of containers, equipment and environment surfaces after cleaning. **BD Difco** D/E Neutralizing Agar will neutralize a broad spectrum of antiseptic and disinfectant chemicals including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. Neutralization allows growth of bacteria which may be held in bacteriostasis after disinfection but are, nevertheless, still viable.

**Side 2** - Coated with **BD Difco** Tryptic Soy Agar, a clear colorless medium recommended in the *European Pharmacopoeia* and *The United States Pharmacopoeia* for microbial limits testing. This medium will give a total aerobic count. The surface under test should be sampled with **BD Difco** Tryptic Soy Agar before cleaning.

#### INCUBATION

Incubate in an upright position at 35–37 °C, examining after 24 and 48 h incubation. Other times and temperature of incubation may be used dependent upon the requirements of the environment tested.

#### READING RESULTS

**BD Difco Tryptic Soy Agar:** Count the number of colonies on the **BD Difco** Tryptic Soy Agar to obtain an assessment of the microbial load before cleaning.

**BD Difco D/E Neutralizing Agar:** Count the number of colonies on **BD Difco** D/E Neutralizing Agar to detect the number of organisms surviving cleaning and sanitization.

#### TYPICAL CULTURAL RESPONSE

Organism	BD Difco Tryptic Soy Agar (24 hours)	BD Difco D/E Neutralizing Agar (24 hours)
<i>Aspergillus niger</i> NCPF 2275	Good growth	Good growth
<i>Bacillus subtilis</i> ATCC 6633	Good growth of large rough colonies	Good growth with medium turning yellow around the colonies
<i>Candida albicans</i> ATCC 2091	Good growth	Good growth
* <i>Escherichia coli</i> ATCC 25922	Good growth of cream moist colonies	Good growth with medium turning yellow around the colonies
* <i>Pseudomonas aeruginosa</i> ATCC 27853	Good growth of colorless colonies	Good growth with medium turning yellow around the colonies
* <i>Staphylococcus aureus</i> ATCC 25923	Good growth of cream/yellow colonies	Good growth of cream/yellow colonies
* <i>Staphylococcus epidermidis</i> ATCC 12228	Good growth	Good growth

\*Cultures listed above may be available as Microtrol (UK) or **Bactrol** Plus (USA) Disks.

#### BD Difco Hyccheck - BD Difco D/E Neutralizing Agar

**Sides 1 & 2** - Coated with **BD Difco** D/E Neutralizing Agar, a purple agar gel for determining the efficiency of sanitization of containers, equipment and environment surfaces after cleaning. **BD Difco** D/E Neutralizing Agar will neutralize a broad spectrum of antiseptic and disinfectant chemicals including quaternary ammonium compounds, phenolics, iodine and chlorine preparations, mercurials, formaldehyde and glutaraldehyde. Neutralization allows growth of bacteria which may be held in bacteriostasis after disinfection but are nevertheless still viable.

#### INCUBATION

Incubate in an upright position at 35–37 °C, examining after 24 and 48 h incubation. Other times and temperature of incubation may be used dependent upon the requirements of the environment tested.

#### READING RESULTS

Count the number of colonies on both sides of the paddle. They should be approximately equal.



#### TYPICAL CULTURAL RESPONSE

Organism	BD Difco D/E Neutralizing Agar (24 hours, 35–37 °C) Sides 1 & 2
<i>Aspergillus niger</i> NCPF 2275	Good growth
<i>Bacillus subtilis</i> ATCC 6633	Good growth of large rough colonies
<i>Candida albicans</i> ATCC 2091	Good growth
* <i>Escherichia coli</i> ATCC 25922	Good growth of cream moist colonies
* <i>Pseudomonas aeruginosa</i> ATCC 27853	Good growth, colorless colonies
* <i>Staphylococcus aureus</i> ATCC 25923	Good growth, cream/yellow colonies
* <i>Staphylococcus epidermidis</i> ATCC 12228	Good growth
*Cultures listed above may be available as Microtrol (UK) or <b>Bactrol</b> Plus (USA) Disks.	

#### BD Difco Hycheck - Total Count

Both paddle sides have **BD Difco** Plate Count Agar surfaces but one agar also incorporates 0.01% triphenyl tetrazolium chloride (TTC). This aids the recognition of microorganism colonies due to the formation of the insoluble red formazan dye when the TTC is reduced.

#### INCUBATION

Incubate in an upright position at 35–37 °C, examining after 24 and 48 h incubation. Other times and temperature of incubation may be used dependent upon the requirements of the environment tested.

#### READING RESULTS

Count the number of colonies on both sides of the paddle. They should be approximately equal. A few microorganisms may grow less profusely in the presence of TTC. If the colony count is not equivalent on the two surfaces then take the larger count as representing the microbial load from the sample under test.

#### TYPICAL CULTURAL RESPONSE

Organism	BD Difco Plate Count Agar (24 hours)	BD Difco Plate Count Agar with TTC (24 hours)
* <i>Enterococcus faecalis</i> ATCC 19433	Good growth	Good growth
* <i>Escherichia coli</i> ATCC 25922	Good growth of cream moist colonies	Good growth, red dye reduction
* <i>Proteus vulgaris</i> ATCC 13315	Good growth	Good growth
* <i>Salmonella typhimurium</i> ATCC 14028	Good growth	Good growth
* <i>Staphylococcus aureus</i> ATCC 25923	Good growth of cream/yellow colonies	Moderate growth, red dye reduction
*Cultures listed above may be available as Microtrol (UK) or <b>Bactrol</b> Plus (USA) Disks.		

#### LIMITATIONS

Do not use the **BD Difco Hycheck** Slide if it is contaminated or the agar medium is significantly dehydrated. The "Typical Cultural Response" tables indicate the expected reactions with various control strains of organisms.

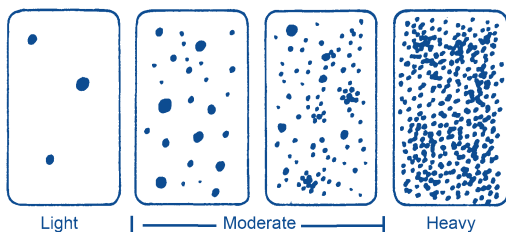
## AVAILABILITY

We are happy to customize **BD Difco Hycheck** Slides to individual requirements for a minimum number of 600 slides.  
Technical Information: In the United States contact BD Technical Service and Support at 1.800.638.8663 or [www.bd.com](http://www.bd.com).

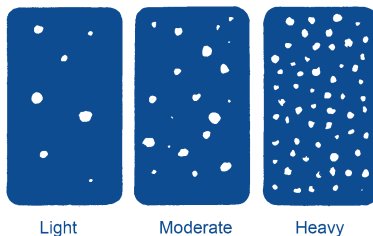
<b>Cat. No.</b>	<b>Description</b>	<b>Pack Size</b>
<b>290001</b>	<b>BD Difco™ Hycheck D/E Neutralizing</b> (D/E Neutralizing Agar both sides)	10 units
<b>290002</b>	<b>BD Difco™ Hycheck Disinfection Control</b> (Tryptic Soy Agar and D/E Neutralizing Agar)	10 units
<b>290003</b>	<b>BD Difco™ Hycheck Enterobacteriaceae</b> (Tryptic Soy Agar and Violet Red Bile Glucose Agar)	10 units
<b>290004</b>	<b>BD Difco™ Hycheck Plate Count Agar with TTC</b> (Plate Count Agar with TTC both sides)	10 units
<b>290005</b>	<b>BD Difco™ Hycheck Total Count</b> (Plate Count Agar and Plate Count Agar with TTC) Primarily for control of Cutting Oils and Process Water.	10 units
<b>290006</b>	<b>BD Difco™ Hycheck Yeasts and Molds</b> (Tryptic Soy Agar and Rose Bengal Chloramphenicol Agar)	10 units
<b>290007</b>	<b>BD Difco™ Hycheck Yeasts and Molds with TTC</b> (Tryptic Soy Agar with 0.01% TTC and Rose Bengal Chloramphenicol Agar)	10 units

## HYGIENIC ASSESSMENT OF SURFACES

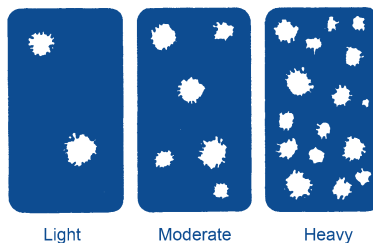
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**YEASTS** (290006, 290007)



**MOLDS** (290006, 290007)

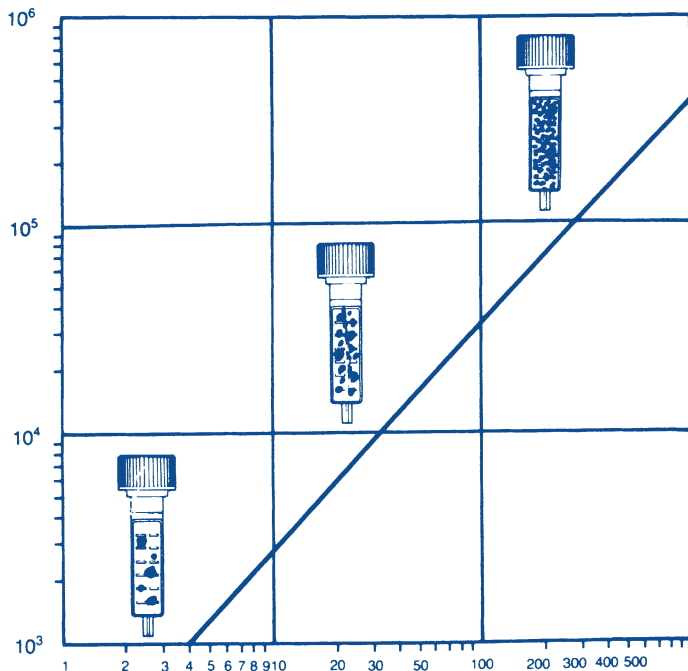


This chart is intended as a guide only; users should always validate their own application.

Microorganism counts with **BD Difco Hycheck** are a semi-quantitative guide to the degree of contamination in the environment being tested.

Monitoring with **BD Difco Hycheck** over a period of time gives a good indication of change in the microbial load at a given site.

**HYGIENIC ASSESSMENT OF  
LIQUIDS**  
**Median Regression Slope (non-selective media)**



Bacterial Colonies per **BD Difco Hycheck** Contact Slide

This chart is intended as a guide only; users should always validate their own application.

Microorganism counts with **BD Difco Hycheck** are a semi-quantitative guide to the degree of contamination in the environment being tested.

Monitoring with **BD Difco Hycheck** over a period of time gives a good indication of change in the microbial load at a given site.

## REFERENCES

1. Restaino, L. 1994. Hycheck Slides versus contact plates compared to the swab technique. Dairy, Food and Environ. sanit. 14:528 - 530.
2. Scott, E. Bloomfield, S.F., and Barlow, C.G. (1984). "A comparison of contact plate and calcium alginate swab techniques for quantitative assessment of bacteriological contamination of environmental surfaces." Journal Applied Bacteriology, 56:317 - 320.
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6. Cain, R.M. and Steele, H. (1953). "The use of calcium alginate soluble wool for the examination of cleansed eating utensils." Canadian Journal of Public Health, 44:464 - 467.

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7 Loveton Circle  
Sparks, MD 21152 USA

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