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HygieneChek™

for
Microbiological
Hygiene Monitoring

Version: 3 (0419)

Please read the manual thoroughly!

Table of contents

HygieneChek™

1. Introduction

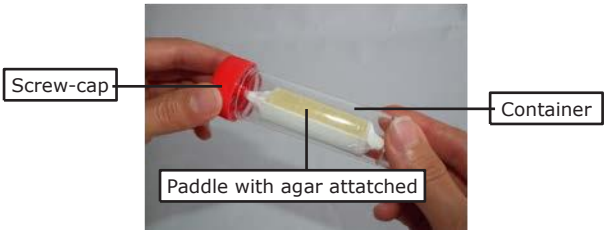
The EU-Regulation No. 852/2004 on hygiene of foodstuffs requires food business operators to assess cleaning practices and establish hygiene procedures according to HACCP principles. The main objective is to safeguard the quality of products and protect consumer health.

According to the ISO 18593:2018-10, contact slides like the **HygieneChek™** can be used by the food sector to estimate microorganisms on surfaces. Easy to handle, it can be used to grade microorganism counts and establish trends in microbiological contamination. Consequently, hygiene monitoring results are benchmarked in compliance with implemented hygiene guidelines.

In addition, the **HygieneChek™** system ensures that samples are transported safely under prevailing temperature conditions.

2. Test principle

The **HygieneChek™** system consists of a container with screw-cap and a double-sided plastic paddle containing two agars of choice.



Sampling is done by pressing the paddle with the agar on a surface, or/ and by dipping into a liquid or liquid emulsion. Alternatively, the agar can be inoculated with a swab. For air sampling, simply place the sample in an upright position on the screw-cap. With **HygieneChek™**, samples can also be sent to an external lab for further investigation.

3. Specificity and test duration

- The specificity of the media for **HygieneChek™** determines which microorganisms are assessed (see DIN 10113-3, Beuth-Verlag).
- For liquid-testing, the limit of detection is 10^2 colony forming units/ml (cfu/ml).
- The duration of the test depends on individual requirements for the microorganisms tested.

4. Kit contents

HygieneChek™ is shipped as a box of 20 paddles. Each paddle is coated on both sides with agar, for a total of 40 tests.

5. Additional equipment required

- Incubator 25 – 37 °C
- Autoclave/Autoclave bags
- Sterile diluents (e. g. ringer)

6. General remarks

Please follow the storage instructions on the packaging.
Do not touch the agar surface! To remove the screw-cap from the paddle, please twist it slightly. To ventilate, open the cap in a 3/4 turn. The container should be tightly closed with the screw-cap before transporting.
After obtaining the results, dispose the paddles by autoclaving (121 °C, 2 bar, 30 min).
We also recommend seeking help from a microbiological lab if **HygieneChek™** is to be used to monitor in-house hygiene.

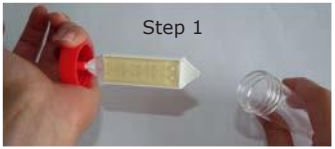
7. Sample preparation

Solid, semi-solid samples or high viscous liquids have to be diluted 1:10 or 1:20 using sterile isotonic saline or ringer's.
Please note: Always consider the dilution factors when calculating the results. For liquids with low viscosity, no dilution is required. The surfaces of meat and fish can also be tested directly.

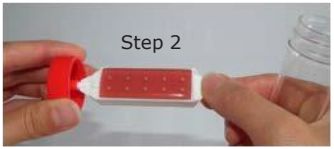
8. Test procedure

a) Surface sampling

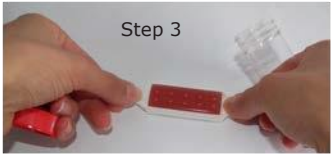
Classic Method



Open the screw-cap. Take the paddle out of the container. Please be careful to hold the paddle with your left hand on the cap and do not touch the agar. Keep the container in your right hand.

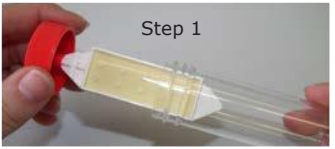


Hold the paddle with the right hand on the terminal end between thumb and index finger. With the left hand, remove the cap by twisting it slightly.

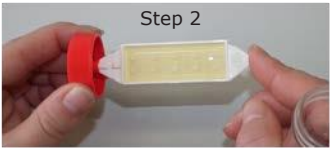


Keep cap and container in your hands as being shown and hold with the left hand the stick of the paddle. Hold the terminal side of the paddle with your finger tip of your right hand and press the paddle firmly onto the surface. Do not wipe with the agar over the surface!

Alternative Method



Unscrew the container and take out the paddle. Don't touch the agar!



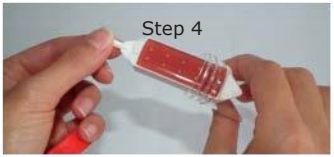
Hold the paddle on one side and the cap on the other side with your finger tip.

Kink the cap and press firmly with both hands, but taking care not to put too much pressure on the surface.

Do not press the agar onto the surface by use of only one hand!

HygieneChek™

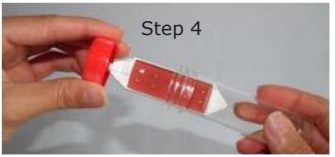
Classical Method



Hold the paddle with your left hand on the stick and place it back into the container. Screw the red cap back onto the container.

To avoid a mix-up when paddles with the same agar on both sides are used, the paddle tip is marked on one side with the recycling sign and on the other side with a number from 1 to 4.

Alternative Method



Place the paddle back into the container and close it tightly. Label the container with a sticker showing the location, time and other sample data.

b) Liquid testing

Unscrew the cap. Hold the paddle on the screw-cap and immerse the agar fully in the test liquid. The agar of the paddle has to be totally covered by the suspension or liquid. Drain off any excess liquid and remaining droplets on the terminal tip at the rim of the beaker glass or on absorbent paper.

c) Staff hygiene

Hands should be tested frequently to ensure that they have been properly cleaned and disinfected by washing. For this, perform tests of the surfaces of fingers, skin and white coats.

d) Air sampling

To collect airborne bacteria, spores, molds and yeast, put the paddle upright on the cap for a minimum of 30 min.

e) Other options

The agar can be inoculated with swabs. The paddle can be used to spread colonies. Single colonies can also be picked from the agar for further differentiation.

9. Incubation

Open the screw-cap in a 3/4 turn to ventilate. Depending on the agar combination, the following incubation temperatures are recommended.

Medium	Incubatin temp.	First reading of results after	Final results after
Total Count	30 – 35 °C	24 hours	48 hours
TTC Total Count	30 – 35 °C	24 hours	48 hours
Coliforms	35 – 37 °C	24 hours	48 hours
Enterobacteriaceae	35 – 37 °C	24 hours	48 hours
Lactic acid bacteria	30 °C	24 hours	48 hours
Yeasts & molds	20 – 30 °C	48 hours	120 hours
Staphylococcus	35 – 37 °C	24 hours	36 hours
ALOA *Listeria*	35 – 37 °C	24 hours	48 hours
CHROMagar *Salmonella*	35 – 37 °C	24 hours	48 hours
CHROMagar *E. coli*	35 – 37 °C	24 hours	48 hours
CHROMagar *S. aureus*	35 – 37 °C	-	24 hours

10. Results

Total count (Agar is beige)

The medium does not contain any neutralizing agents and supports the growth of all bacteria, yeasts and molds. Colonies appear in individual color and size.

Lactic acid bacteria (Agar is honey colored)

The medium is applied to detect hetero-fermentative lactic acid bacteria. However, the medium is not selective for lactic acid bacteria. This means that lactic acid producing *Staphylococcus* spp. and other bacteria may also grow on the thiamine-rich medium.

Coliforms VRBL (Agar is bordeaux colored)

The medium is used for the selective detection of Coliforms, including *E. coli*. Coliforms (*Escherichia, Enterobacter, Klebsiella,* and *Citrobacter*) can metabolize lactose in the medium. They appear as **red violet to mauve** colonies. All lactose-negative (which means non-Coliform) Enterobacteriaceae appear **colorless**.

Enterobacteriaceae VRBD (Agar is reddish brown)

The medium is used for the selective detection of Enterobacteriaceae. Enterobacteriaceae metabolize glucose in the medium and appear as **pink**, **violet** or **violet-red** colonies. Colorless colonies are not members of the Enterobacteriaceae family.

Yeasts & Molds (Agar is light brown)

The medium is used for the selective detection of yeasts & molds. Yeasts appear as small **white brown** colonies with a well defined outline. Molds grow in filamentous shape. Colonies may be colored. They spread rapidly over the agar surface (looks like cotton flakes) and can occupy the whole container. In this case, do not open the container to avoid the spreading of fungal spores.

HygieneChek™

***Staphylococcus* (Agar is pink red)**

The medium is used for the selective detection of pathogenic *Staphylococcus*. Mannit-positive bacteria show a **yellow** halo around the colonies. Sometimes the whole agar shows a yellow color. Colonies appear in a **white yellow** color showing different sizes.

CHROMagar *Salmonella* (Agar is pink)

The medium is used for the selective detection of Salmonella and corresponds to RAMBACH medium. *Salmonella* metabolize propylene glycolic acid, which makes the colonies appear in a **red** color. Coliforms appear in a **blue green** up to **blue violet** color. Other Enterobacteriaceae and gram-negative bacteria grow **colorless** or in a **yellowish** color. **Please apply CHROMagar *Salmonella* only as a contact-slide for surface testing.**

TTC Total count (Agar is white yellow)

Colonies appear in a **red** color or show a red dot in the centre of the colonies. All red colonies are counted. The final reading of the results should be done 72 hours after incubation. The growth of *Staphylococcus* and yeasts may be reduced on TTC-containing medium.

Disinfection control (Agar is violet)

The medium is used for the detection of total counts upon cleaning and sanitation. The growth of microorganisms may cause the medium to turn yellow. For molds, it remains pink. The **colours of the medium and colonies are not taken into consideration when counting the colonies**. **Each colony is counted**.
Do note that as microorganisms are often harmed by cleaning and disinfection, it may take longer for them to appear on the agar. Please check the paddles laterally to better detect the colonies. Read the final results 48 hours after incubation.

CHROMagar *E. coli* (Agar is beige)

ß-Glucuronidase-positive *E. coli* appear in **blue** colonies. All other Coliforms or Enterobacteriaceae appear **white** or **colorless**.

ALOA *Listeria* (Agar is yellow)

Due to the supplement of Lithium chloride and different antibiotics, the medium is selective for the detection of *Listeria*. *Listeria* colonies appear in a **light-blue** to **turquoise** color, caused by different indicators in the medium.

CHROMagar *Staphylococcus aureus* (Agar is light yellow)

The medium is used for the detection of *Staphylococcus aureus*, which is known for the ability to produce Staphylococcal Enterotoxin (SET). The colonies of *Staphylococcus aureus* appear **mauve pink**. Others appear **blue** or **colorless**.

CHROMagar Mastitis

The evaluation of the results when using CHROMagar Mastitis is explained in detail in a special application note.

11. Interpretation of results

To better detect colorless transparent colonies, please also check the paddles laterally.

a) Colony counting upon surface testing and air sampling

1. Assess the number of colonies (by counting or estimation)
2. There are several ways to record the results:
   - Divide the number of colonies by 9. This is the total number of microorgansims tested with regard to the surface of the paddle (9 cm^2). The result is reported as cfu/cm^2.
   - Due to repeated testing, the number of colonies on the paddle for a sample area should be determined.
   - We suggest the gradation of the cfu in the following categories:

Number of colonies on the total count medium: cfu/cm^2	Remarks	Category
0 – 10	Very slight to moderately contaminated, still acceptable	1
>10	Not acceptable	2

Number of colonies on the Entero-bacteriaceae medium: cfu/cm²	Remark	Category
0 – 1	Very slight to moderately contaminated, still acceptable	1
>1	Not acceptable	2

b) Colony counting for liquid sampling:

The limit of detection is 10^2 cfu/ml. This means that at least 100 microorganisms have to be present in 1 ml of an liquid sample before any colonies appear on the paddle. Please compare the paddle with the Result Interpretation Guide (on the back side of this package insert and also as separate sheet available) to assess the microbiological status of the liquid.

12. Media available – Names, Color

Art. No.	Name (Combinations)		Agar color	
10001644	Total count	Total count	beige	beige
10001645	Total count	Lactic acid bacteria	beige	honey yellow
10001646	Total count	Coliforms	beige	bordeaux
10001647	Lactic acid bacteria	Yeasts & Molds	honey yellow	light brown
10001648	Total count	Yeasts & Molds	beige	light brown
10001649	*Staphylococcus*	*Staphylococcus*	fuchsia-red	fuchsia-red
10001650	Yeasts & Molds	Yeasts & Molds	light brown	light brown
10001651	Coliforms	Coliforms	bordeaux	bordeaux
10001652	Coliforms	Yeasts & Molds	bordeaux	light brown
10001653	TTC-Total count	Yeasts & Molds	white-yellow	light brown
10001654	TTC-Total count	Coliforms	white-yellow	bordeaux
10001655	TTC-Total count	TTC-Total count	white-yellow	white-yellow
10001656	Disinfection control	Disinfection control	purple	purple
10001657	CHROMagar *E. coli*	Coliforms	beige	bordeaux
10001658	CHROMagar *Salmonella*	CHROMagar *Salmonella*	pink	pink
10001659	ALOA *Listeria*	ALOA *Listeria*	yellow	yellow
10001660	Total count	Enterobacteriaceae	beige	red-brown
10001661	CHROMagar *S.aureus*	CHROMagar *S.aureus*	light yellow	light yellow
10001662	Enterobacteriaceae	Enterobacteriaceae	red-brown	red-brown
10001664	CHROMagar Mastitis gram +	CHROMagar Mastitis gram –	white opaque	transparent amber

13. Media available – Composition

Item No.	Composition
10001644	Plate-Count-Agar/Plate-Count-Agar
10001645	Plate-Count-Agar/APT (All-Purpose-Medium with tween)
10001646	Plate-Count-Agar/Violet Red Bile Agar
10001647	APT (All-Purpose-Medium with tween)/Oxytetracyclin-Glucose-Yeast
10001648	Plate-Count-Agar/Oxytetracyclin-Glucose-Yeast
10001649	Mannit-sodium chloride-phenol red-Agar/Mannit-sodium chloride-phenol red-Agar
10001650	Oxytetracyclin-glucose-Yeast/Oxytetracyclin-glucose-Yeast
10001651	Violet Red Bile Agar/Violet Red Bile Agar
10001652	Violet Red Bile Agar/Oxytetracyclin-Glucose-Yeast
10001653	Plate-Count-Agar + 0,01 %TTC/Oxytetracyclin-Glucose-Yeast
10001654	Plate-Count-Agar + 0,01 %TTC/Violet Red Bile Agar
10001655	Plate-Count-Agar + 0,01 %TTC/Plate-Count-Agar + 0,01%TTC
10001656	Dey-Engely Neutralizing Agar/Dey-Engely Neutralizing Agar
10001657	Selective agar for Enterobacteriaceae with ß-glucuronidase-indicator/Violet Red Bile Agar
10001658	Rambach-Agar/Rambach-Agar
10001659	ALOA *Listeria* Agar/ALOA *Listeria* Agar
10001660	Plate Count Agar/Violet Red Bile Dextrose Agar
10001661	Selective agar for *Staphylococcus aureus* with color indicator
10001662	Violet Red Bile Dextrose Agar/Violet Red Bile Dextrose Agar
10001664	Selective agar for Mastitis, one side for gram + strains, the other side for gram - strains

14. Media available – Storage

Item No.	Code	Storage*
10001644	DS 004	15 – 25 °C
10001645	DS 005	15 – 25 °C
10001646	DS 006	15 – 25 °C
10001647	DS 010	15 – 25 °C
10001648	DS 012	15 – 25 °C
10001649	DS 013	15 – 25 °C
10001650	DS 016	15 – 25 °C
10001651	DS 017	15 – 25 °C
10001652	DS 021	15 – 25 °C
10001653	DS 023	15 – 25 °C
10001654	DS 024	15 – 25 °C
10001655	DS 026	15 – 25 °C
10001656	DS 028	15 – 25 °C
10001657	DS 035	2 – 15 °C
10001658	DS 036	2 – 15 °C
10001659	DS 041	2 – 15 °C
10001660	DS 046	15 – 25 °C
10001661	DS 049	2 – 15 °C
10001662	DS 057	15 – 25 °C
10001664	AP 016	2 – 15 °C

*) Shipping Conditions: 2 – 25 °C ; shipped and stored in darkness

HygieneChek™

15. Accessories for HygieneChek™

Thermocult, mini-incubator.

16. Disclaimer

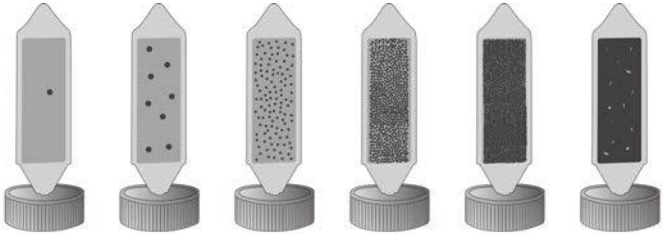
The contents of this package insert is correct to the best of our present knowledge. It aims to infom about the product and its appropriate usage. It, however, does not guarantee certain properties or usage fields of application.

Microbiology

HygieneChek™ Result Interpretation Guide

Dipping into liquids

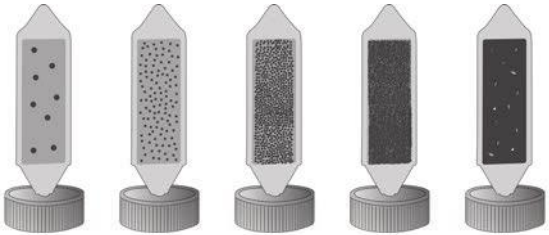
The limit of detection is 10^2 cfu/ml. This means that at least 100 microorganisms have to be present in 1 ml of liquid sample before any colonies appear on the paddle. Compare your paddle with the pictures below for estimated values.



10^2 10^3 10^4 10^5 10^6 10^7

Surface contact

For the evaluation of surface contact tests, count the colonies and divide the results by 9 to get the cfu/cm², as the area of one paddle side is 9 cm². Or just compare it with the pictures below. For selective agar HygieneChek™ products, count the colonies as specified in the package insert.



1 cfu/cm² 10 cfu/cm² 50 cfu/cm² 250 cfu/cm² > 250 cfu/cm²

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