Making the World's Food Safer

HygieneChek™ PLUS

Dip-Slides for Microbiological Monitoring

INSTRUCTIONS FOR USE

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www.romerlabs.com

1. Intended purpose

HygieneChek™ PLUS dip-slides are semi-quantitative, culture-based analytical tools designed for the monitoring of microbial contamination in the food chain, as well as other sectors that require the control of microorganisms. The dip-slides are ready-to-use, and the testing protocol requires little expertise to execute. Unlike quantitative cultures, testing with dip-slides can be carried out regardless of access to a microbiology laboratory. In addition, the design of the dip-slides ensures that recovered microorganisms are safely contained, thus preventing the accidental contamination of facilities.

Although most sample types can be tested with the HygieneChek™ PLUS dip-slides, surfaces and liquids are the most appropriate. Flat surfaces and non-viscous liquids can be tested through direct inoculation, whereas other sample types require sampling or sample preparation steps to be carried out before inoculation onto the dip-slides. Non-flat surfaces require sampling using a swab; other sample types, such as viscous liquids, require prior dilution.

Though a semiquantitative technique, testing with dip-slides produces results that are comparable in magnitude to quantitative methods employing agar plates. Therefore, HygieneChek™ PLUS dip-slides can be used to estimate microbial counts and observe trends in contamination in compliance with implemented monitoring guidelines. If no change in contamination patterns is observed, the dip-slide may be discarded to avoid further time-consuming and costly workup. Alternatively, it may be sent to a competent microbiology laboratory for further investigation.

2. Package contents

HygieneChek™ PLUS dip-slides are sold in boxes of 10. Each slide is covered on both sides with an agar culture medium (see Table 1), either the same or different (please consult the product inlay, the catalogue or the Romer Labs Webshop for a list of available combinations), for a total of 20 tests.

3. Storage conditions

Most of the agars can be stored at 2 to 25 °C. However, dip-slides containing AL and TBX should not be stored at temperatures above 8 °C. Appropriate storage temperatures are printed on the labels applied to boxes. Large and frequent fluctuations in temperature during storage and transportation can cause water to evaporate from the agar and condense in the tube container, which eventually can lead to dehydration of the agar. Storage in a frequently opened refrigerator can cause such fluctuations to occur. A small amount of condensate is normal and will not influence test performance. Dip-slides removed from the box should be stored in a manner that prevents exposure to light.

4. Equipment and consumables

Testing with the HygieneChek™ PLUS dip-slides will require the following:

- Microbiological incubator, capable of operating in the temperature range specific to the tested parameter (see point 9);
- Sterile beaker or other container, 80 mm deep (for liquid samples);
- Autoclave and autoclave bags, or other means of inactivating or safely confining biohazardous material.

5. Test principle

Testing with the HygieneChek™ PLUS dip-slides follows a three-step procedure: (1) surface inoculation of the agars by one of several techniques, according to sample type; (2) incubation of the dip-slides at the required temperature, from 1 to 10 days, depending on the microorganisms of interest; and (3) assessment of microbial growth and expression of results. A fourth step is normally required when the presence of pathogenic *Salmonella* spp., *Listeria* spp. or *Staphylococcus* spp. is suspected, which involves the shipment of the dip-slides containing presumptive colonies to a competent microbiology laboratory for confirmation.

Table 1. *Agar media used for HygieneChek™ PLUS dip-slides*

Parameter	Agar name	Formula[1]	Color[2]	Incubation regime[3,4]	
Total Count TTC	Tryptone Soy Agar (TSA) + TTC[5]	ISO 22964	cream/amber	30-37 °C	24-72 *h*[6]
Yeasts & Molds	Malt Extract Agar (MEA)	EN 1650	amber	25-30 °C	3-7 *d*[6]
Enterobacteriaceae	Violet Red Bile Glucose Agar (VRBG)	ISO 21528	red/violet	30-37 °C	18-24 *h*
Coliforms	Violet Red Bile Lactose Agar (VRBL)	ISO 4832	red/violet	30-37 °C	18-24 *h*
E.coli	Tryptone-Bile-X-Glucuronate Agar (TBX)	ISO 16649	cream/white	37-44 °C	20-24 *h*
Disinfection Control	Dey-Engley Neutralizing Agar (DEA)	USP[7]	purple/violet	30-37 °C	24-48 *h*
Lactic Acid Bacteria	Agar acc. de Mann, Rogosa & Sharpe (MRS)	ISO 15214	amber/brown	30±1 °C	48-72 *h*
Salmonella	Xylose Lysine Desoxycholate Agar (XLD)	ISO 6579	red	36±2 °C	21-27 *h*
Listeria	Agar Listeria acc. Ottaviani & Agosti (AL)	ISO 11290	cream	37±1 °C	24-48 *h*
Staphylococcus	Mannitol Salt Agar (MSA)	EP[8]; USP	red	36±2 °C	24-48 *h*

[1]As defined in the indicated reference documents; [2]Typical for fresh agars, it may change during incubation as a result of microbial growth; [3]Users should adjust incubation conditions according to scope and process-specific requirements (may require validation); [4]For counts at the upper limit, when longer incubation would result in spreading/overlapping colonies, reading after a shorter incubation time is recommended; [5]TTC – 2,3,5-triphenyltetrazolium chloride; [6]Incubation for total counts and yeasts and molds can also be conducted at 7±1 °C for up to 10 d, when psychrotrophic microorganisms are sought; [7]USP – United States Pharmacopeia; [8]EP – European Pharmacopeia.

The surface of the agar deposited on both sides of the slide is inoculated by one of the following means: (1) direct contact with a surface; (2) dipping into liquids; or (3) streaking with a swab-stick. Incubation is carried out in aerobic conditions, at temperatures and for the duration that will allow the target microorganism to grow. Assessment of microbial growth is carried out by examining and perhaps counting the colonies that emerged on the agar surface. The comparison chart in Annex 3 can be used for a quick estimation of results.

Figure 1. *Components of HygieneChek™ PLUS dip-slides (1 – tube-container; 2 – screwcap-slide; 3 – agar)*

6. General remarks

The design of the HygieneChek™ PLUS dip-slides includes three components: (1) a transparent tube container; (2) a single piece element consisting of a screwcap and a slide; and (3) two agar layers that cover each side of the slide over a surface area of 10 cm^2 (1.55 in^2) per side (Figure 1). Both the tube container and the screwcap-slide element are built from polypropylene (PP), a plastic material that does not break into shards or splinters.

While manipulating the dip-slides, the surface of the agar should not be touched. To open the tube container and remove the slide from the container, twist the screwcap counterclockwise. To close and seal the tube container, twist it clockwise. To ventilate, open the cap by ½. The container should be ventilated while incubating and sealed for all other purposes, especially for transportation.

The delay between sampling/inoculation and incubation should be no longer than 48 *h*. We recommend incubating the dip-slides as soon as possible after inoculation and no later than 24 *h*. If incubation is not possible within 45 minutes from sampling/inoculation, the dip-slides should always be kept cooled at 1 to 8 °C with the container sealed.

7. Testing of surfaces

Microorganisms can be found on visually clean surfaces but are most common in wet and soiled spots where they can persist or even proliferate. Hard-to-clean equipment or difficult-to-reach areas such as holes, gaps and edges are potential microbial reservoirs that should be preferentially sampled. Equipment with otherwise unreachable surfaces where food can accumulate may need to be dismantled. Sampling can be performed either during or after production, as well as after cleaning and disinfection. For general guidance on the testing of surfaces, please refer to ISO 18593.

Flat surfaces are sampled by the agar contact technique (see Annex 1), in which the meniscus-shaped surface of the agar is gently pressed onto the surface to be tested while avoiding gliding, thus retrieving and being inoculated with part of its microbial load. The sampling of surfaces is notoriously uneven. When sampling with the HygieneChek™ PLUS dip-slides, consistency of time (*circa* 10 *s*) and pressure will allow for better accuracy and reproducibility of results. Applying too much pressure will result in an imperfect contact of the agar with the surface to be tested. After sampling, the surface should be wiped using 70% ethanol to avoid traces of nutrients, moisture or other substances remaining on the sampled surface.

Non-flat surfaces and hard-to-reach spots can be tested by sampling with a sterile swab-stick and then by streaking the swab onto the agar surface (see Annex 1). Recovery of microorganisms is higher when moistened swabs are used. If the test area is wet, a dry swab is recommended; if it is dry, then a moistened swab is required.

To monitor personnel hygiene, testing can be performed by either the agar contact technique or the swab technique, depending on the characteristics of the surface to be tested. The agar can be pressed against palms, fingertips, and clothing (see Annex 1). A moistened swab-stick can be used to sample between fingers, on boot soles and other areas that could harbor bacteria.

8. Testing of liquids

Liquids can be tested using the dipping technique (see Annex 1). After sampling and thorough mixing, a subsample should be poured into a sterile beaker – the tube container can also be used for this purpose. The dip-slide must be held by the screwcap handle, while the agar is completely submerged in the test liquid. The volume of test liquid should be large enough to allow for a full immersion. Surplus liquid and any remaining droplets should be drained off by touching the tip of the slide to the inner wall or rim of the beaker, or on absorbent paper. Excess water on the agar surface will favor the development of lawns rather than individual colonies.

Viscous or highly contaminated liquids require dilution with sterile diluent prior to inoculation. Usually, a ratio of 9 parts diluent to 1 part sample is employed (a decimal dilution). Peptone saline, buffered peptone water or other general-purpose diluents are recommended. When performing sample dilution, consideration should be given to the estimated limit of detection of the dipping technique (100 *CFU/mL*). Alternatively, inoculation can be done *via* the swab technique.

9. Incubation

When preparing the dip-slides for incubation, the screwcap should be loosened (opened by ½ turn) to allow for a proper ventilation of the agar. Incubation regimes are chosen according to the agar type (see Table 1). If a combination of two different agar types is used, a temperature suitable for both types should be selected (please also consult the HygieneChek™ PLUS product inlay).

10. Assessment of growth

After incubation, the agars should be inspected for microbial growth (see Annex 2). To better detect colorless and/or transparent colonies, slides should also be checked laterally. If the development of spreading colonies is to be expected, intermediary examinations are recommended. Contamination levels are assessed by either the counting of colonies or by employing the Quick Results Comparison Chart given in Annex 3.

11. Expression of results

Testing of surfaces

Inoculation by the agar contact technique allows for quantitative results, typically expressed in *CFU/cm²* or *CFU/in²*. To achieve this, the number of characteristic colonies should be divided by the size of the sampled area (10 *cm²* or 20 *cm²*, or 1.55 *in²* or 3.1 *in²*, if both sides have the same agar and were used to test the same surface). When interpreting quantitative results, consideration should be given to the fact that recovery rates of agar contact techniques can vary extensively with the surface type and the structure of the microbial community. Consistency in sampling and observing trends over time can overcome this limitation.

The use of the Quick Results Comparison Chart will only allow for semiquantitative results, either expressed categorically (*e.g.*, light, moderate or heavy contamination) or numerically (*e.g.*, 50 *CFU/cm²*). Inoculation *via* streaking with a swab-stick will produce qualitative results.

Testing of liquids

The dipping technique will only allow for semi-quantitative results. The Quick Results Comparison Chart in Annex 3 should be used to assess the contamination level, which can be expressed categorically (*e.g.*, light, moderate or heavy contamination) or as on order of magnitude (*e.g.*, 10^5 *CFU/mL*). Dilution should be factored into the assessment (*i.e.*, multiplication by 10 for decimal dilutions), if performed prior to inoculation. Absence of growth indicates a contamination level below 100 *CFU/mL*. Streaking with a swab-stick will produce qualitative results.

12. Quality control

The HygieneChek™ PLUS dip-slides are filled in a clean-room-like environment, quarantined, and visually checked for contamination before shipping. Nonetheless, contamination is sometimes unavoidable. Therefore, please make sure to check the agar before performing the test. Do not use the product if contamination, agar dehydration or agar detachment are observed. Typically, not all pieces in the box are affected when contamination occurs. Please examine the content of the package with care.

Representative samples of every batch are tested for physical-chemical characteristics, sterility and performance in accordance with standard operating procedures based on ISO 11133:2014. Certificates can be downloaded from Romer Labs Customer Resources using the batch number inscribed on the box.

13. Disposal

The user must ensure safe disposal of used or unusable preparations of this product. Several methods can be employed to inactivate microbial cultures, such as autoclaving, chemical decontamination or incineration. Please consult applicable regulations in your region concerning the collection and disposal of biohazardous material.

References

EN 1650:2019. Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics

ISO 4832:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique

ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection technique

ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

ISO 11290-2:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method

ISO 15214:1998. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of mesophilic lactic acid bacteria — Colony-count technique at 30 °C

ISO 16649-1:2018. Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

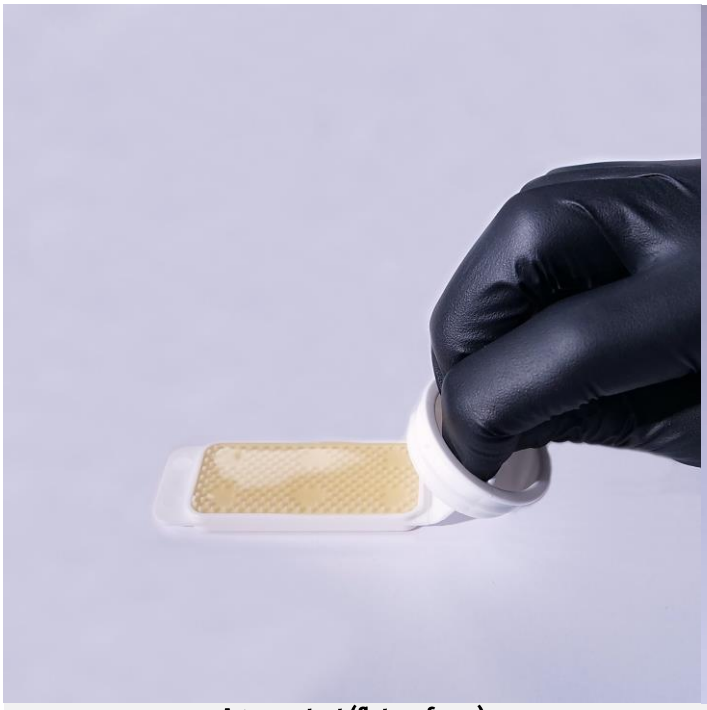
ISO 18593:2018. Microbiology of the food chain — Horizontal methods for surface sampling

ISO 21528-2:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Enterobacteriaceae* — Part 2: Colony-count technique

ISO 22964:2017. Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

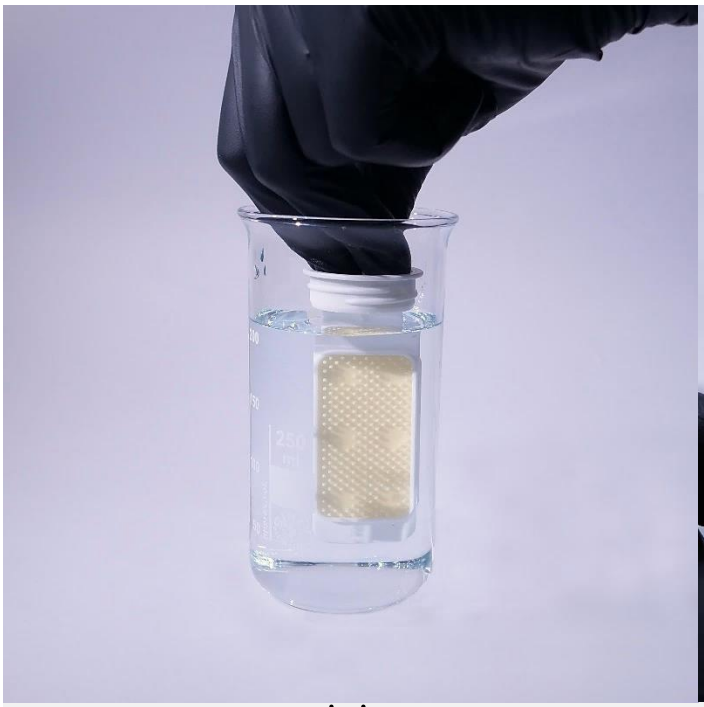
Annex 1

Inoculation Techniques

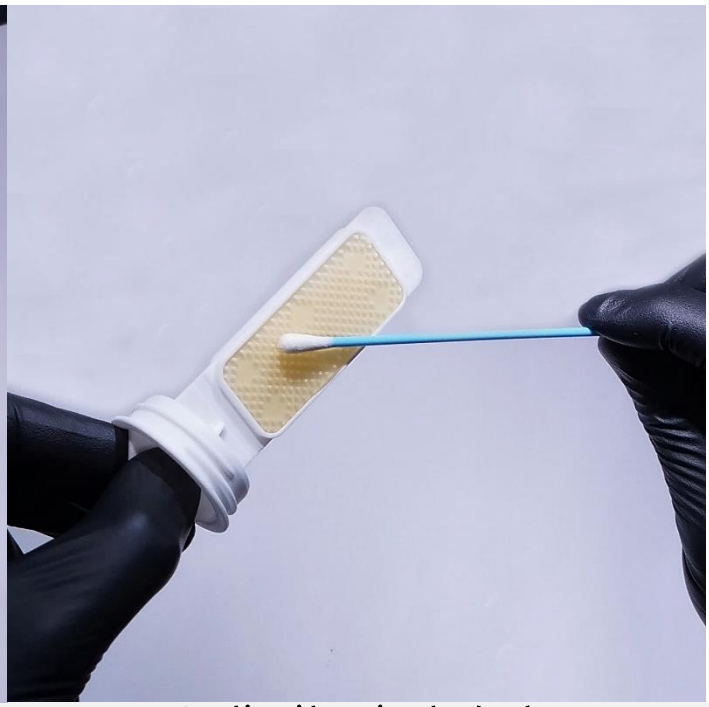


Agar contact (flat surfaces)

Agar contact (personnel hygiene)



Dipping



Streaking with a preinoculated swab

Annex 2

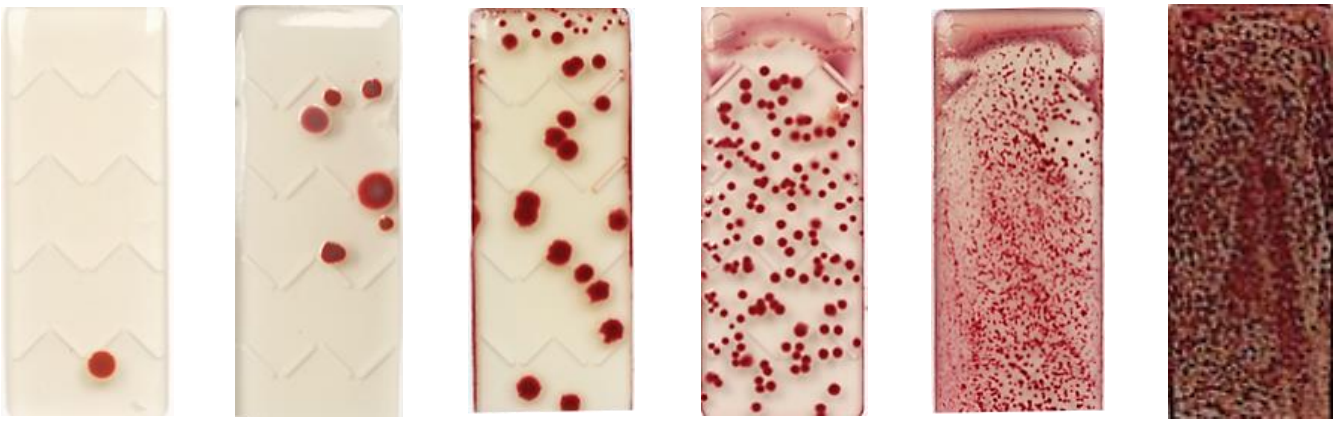
Guide to the examination of colonies developed on HygieneChek™ PLUS dip-slides

Parameter	Agar	Notes
Total Count TTC	TSA + TTC	▪ The agar is clear and cream to amber in color; it may turn yellow if improperly stored (prolonged exposure to light). ▪ The non-selective, general-purpose formula supports the growth of most aerobic or aerotolerant species found in the food chain. ▪ Counts are primarily comprised of bacteria, though incubation times near 72 *h* will also allow for the growth of some yeasts and molds. ▪ Most **colonies appear red** due to the reduction and accumulation of TTC. ▪ TTC has a slightly inhibitory effect which limits the excessive spreading of colonies; some *Staphylococcus* species may require up to 72 *h* to grow. ▪ Areas of the agar may turn red in color after exposure to reducing agents (disinfectants such as aldehydes) or incubation in a tightly closed tube.
Yeasts & Molds	MEA	▪ The agar is clear and amber in color. ▪ **Yeasts produce white to light brown colonies** in under 72 *h*. ▪ **Molds produce fuzzy or grainy colonies** with white margins and intensely colored centers (if sporulation occurs) in 5 to 7 days. Some mold species may take up to 2 weeks to produce visible colonies. ▪ Some (inferior) molds tend to spread rapidly over the agar surface and can occupy the whole container. ▪ Bacteria tolerant lo low *p*H values can generate colonies that could be mistaken for yeasts; if critical, microscopic confirmations are recommended.
Enterobacteriaceae	VRBG	▪ The agar is clear and red to violet in color. ▪ Characteristic **colonies are pink to red or purple** (with or without precipitation halos); certain *Enterobacteriaceae* may cause discoloration of their colonies or of the medium and some may produce spreading colonies. ▪ Definitive results require confirmation of colonies (not critical for monitoring purposes).
Coliforms	VRBL	▪ The agar is clear and red to violet in color. ▪ Characteristic **colonies are purplish red** with a diameter of at least 0.5 *mm* and are sometimes surrounded by a reddish zone; such colonies do not require further confirmation. ▪ Purplish red colonies of smaller size require confirmation for definitive results (not critical for monitoring purposes).
E. coli	TBX	▪ The agar is cream/white in color and slightly opalescent. ▪ The composition of the medium allows for the specific determination without confirmation of β-glucuronidase-positive *Escherichia coli* in the food chain. ▪ **Colonies of** β-glucuronidase-positive ***E. coli* are blue to blue green**. ▪ Some strains of *E. coli*, including pathogenic ones (notably those belonging to serotype O157:H7), will not be detected by this method. ▪ β-glucuronidase activity may also be exhibited at 44 *°C* by certain other members of the *Enterobacteriaceae*, notably *Shigella* spp. and *Salmonella* spp.
Disinfection Control	DEA	▪ The agar is purple to violet in color and may have a fine precipitate. ▪ Sodium thioglycolate, sodium thiosulphate, sodium bisulphite, soya lecithin and polysorbate 80 in the medium will neutralize traces of disinfectants, including quaternary ammonium compounds, phenolic, iodine and chlorine preparations, mercurial, formaldehyde and glutaraldehyde. ▪ The non-selective formula supports the growth of most aerobic or aerotolerant species found in the food chain, though the short incubation time will allow few microorganisms other than bacteria to grow. ▪ Most **colonies will be surrounded by a yellow discoloration zone**; fungi are generally not.
Lactic Acid Bacteria	MRS	▪ The agar is clear and amber to light brown in color. ▪ Characteristic **colonies are white** and at least 0.5 *mm* wide. ▪ Some *Leuconostoc* spp. may form large, slimy colonies that may hinder the development of other colonies. ▪ Definitive results require confirmation of colonies (not critical for monitoring purposes).

Parameter	Agar	Notes
Salmonella	XLD	▪ The agar is clear and red. ▪ Typical ***Salmonella* spp. colonies have a black center** and a lightly transparent margin of reddish color. ▪ Atypical *Salmonella* spp. colonies are pink with a darker pink center, or yellow with or without blackening. ▪ All growth should be presumed to be *Salmonella* spp.; definitive results require confirmation of colonies.
Listeria	AL	▪ The agar is cream in color and slightly opalescent. ▪ **Blue-green colonies** surrounded by an opaque halo are typical for *Listeria monocytogenes* and *Listeria ivanovii*. ▪ Blue-green colonies without a halo are characteristic to other *Listeria* species; some *L. monocytogenes* strains may be slow in developing a halo. ▪ Some *L. monocytogenes* strains may grow very fast, leading to adjoining/overlapping colonies; examination of the agar after only 24 *h* of incubation will aid counting. ▪ All blue-green colonies should be presumed to be *L. monocytogenes*; definitive results require confirmation of colonies.
Staphylococcus	MSA	▪ The agar is clear and red. ▪ Pathogenic **staphylococci form bright yellow colonies**, surrounded by a yellow ring or area. ▪ Non-pathogenic staphylococci generally produce small red colonies which do not change the color of the medium. ▪ After 48 *h*, other species may grow. ▪ Definitive results require confirmation of colonies (not critical for monitoring purposes).

Annex 3

Quick Results Comparison Chart



<10^3 CFU/mL	10^3 CFU/mL	10^4 CFU/mL	10^5 CFU/mL	10^6 CFU/mL	10^7 CFU/mL
<1 CFU/cm^2	1 CFU/cm^2	10 CFU/cm^2	50 CFU/cm^2	250 CFU/cm^2	>250 CFU/cm^2
Light		**Moderate**		**Heavy**	

B a c t e r i a

Light	Moderate	Heavy	Light	Moderate	Heavy
Y e a s t s			**M o l d s**		