Frequent Q & A on Sanita-kun

Chisso Corporation

	Questions	Answers	Memo
A) Application-Incubation			
1	There are some wrinkles on a sheet, when I applied. Any trouble？	No, they don't disturb in the detection. Never smooth up the wrinkles with your fingers. Most of wrinkles will disappear during incubation.	
2	There is an area, diameter around 5 mm, where the sample solution is not absorbed on. Any trouble?	It doesn't disturb in the detection. The area will disappear, if you tap slightly the sheet through a cover film.	
3	There is an opening on the sheet. I failed to adhere the cover film on a sheet, when I put it.	Remove the air by rubbing around the sheet with your finger. Small air doesn't disturb the detection.	
4	How many sheets can I pile up?	All Sanita-kun products, except Y&M, can be pile up to 20～30 sheets. In Y&M, you had better results, if you don't pile them up	This is attributed to the aerophilicity of Yeasts and molds.
5	Viscous solution was leaked from the sheet The shape of the colony was smeared.	Since highly viscous materials are used, the following factors, which will affect a viscosity, make a leakage of the material from the sheets, and make an abnormal shape of colonies. 1. Usage of water in sample dilution. 2. High incubation temperature, >37℃. 3. Too much sample volume, >1.05 mL.	1. PBS or 0.9% NaCl is recommendable for the dilution. (If you must use water, apply 0.9 mL or 1.0 mL of sample solution and incubate at 35℃ or 30℃, respectively.) We adopted 0.9% NaCl for the dilution in the AOAC RI validation. 2. Micropipette is recommendable for the sample application.
6	The surface of the sheet was dry and there was no colony on the sheet.	The sample volume was too small. <0.95mL, or there might be a big air between the cover film and the sheet.	Refer 5. Micropipette is recommendable for the sample application.
7	Diffused colonies or comet-like colonies were observed.	Count a continuously diffused colony as 'one' colony.	Highly motile microorganisms often make such diffused colonies.
B) Sample solutions			
1	What is inappropriate for the sample solution?	1. Highly salty sample High concentration of salt inhibits a growth of microorganisms. 2. Colored sample It is difficult to recognize colonies in a colored background.	Dilute the samples well with PBS of 0.9% NaCl.
2	Can I use water without salt as for the sample dilution？	Refer A)5 and A)7.	Refer A)5 and A)7.

3	Entire sheet was colored in Sanita-kun Coliform, Y&M and Staph. aureus. Why?	Colorization with enzymes in raw meat or vegetables may be observed in an entire sheet. As this will appear in several hours, you can differentiate colonies from background color.	More than 10-times dilution is recommendable, if you mind the background colorization.
c) Miscellaneous			
1	Stock temperature？	Stock them in a refrigerator or a cool and dark site at 2～15℃. Do not use them, if you recognize a color on them.	As Sanita-kun products are dry media, they are stable at room temperature in a short time. We, however, cannot guarantee their quality.
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D) Total Count			
1	Which microorganisms cannot be detected with the Sanita-kun Total Count？	Following microorganisms are not easy to detect with the Sanita-kun, according to our research. 1. Many varieties of lactic acid bacteria. 2. Some varieties of Gram (+) bacteria such as Micrococcus. 3. Some species of Pseudomonas. 4. Thin or partial colorization might be recognized, if you apply too many microorganisms, $>10^6$ cfu/mL.	1. Because, they are aerophobic or they have low activity of TTC reduction. 2. Because, TTC inhibits their growth. 3. Low TTC reduction or growth inhibition by TTC. 4. Competency.
2	Giant colonies?	Some species of mold make rarely gigantic colonies on the TC sheet. You should count a gigantic colony as 'one'.	In such cases with agar plates, you cannot count the colonies because the colonies cover entire surface completely.
E) Coliforms			
1	What merit does the RIDA Coliform Count have?	We adopted sensitive X-gal method as a detection technology.	X-gal is a substrate of β-galactosidase.
2	Colonies were increased after 2-day incubation against the instruction manual.	Injured cells might be detected with more than 24 hr incubation.	
3	Colorization on entire surface?	1. Refer to B)3. 2. Too many lactic acid bacteria, $>10^6$ cfu/mL, in a sample make the entire sheet colorize in a short time (ca. 5 hr) with their enzymes. They make no colonies.	1. Refer to B)3. 2. When you examine a sample such as plain yogurt or raw cheese, you should confirm if there is not an entire colorization after 5 hr incubation. If there is color, you should examine again with more dilution.
4	Yellow colonies?	You should not count yellow colonies.	
5	Green colonies?	You should count green colonies. Some of them may not be coliforms. But you have to be very careful.	Weak enzyme activity results green colonies.
6	No colonies with sugar rich sample?	Rich glucose in sample inhibits the generation of the enzyme in coliforms.	More than 10-times dilution is recommendable.

F) Y & M			
1	Can I differentiate yeasts or molds with RIDA Y&M Count?	Molds make characteristic diffuse colonies. But, precise differentiation may be difficult.	
2	Incubation time?	Usually, we recommend 3 days incubation. Growth of Rhizopus, however, is rapid and entire surface will be covered with them after 3 days incubation. You have to check the sheet after 2 days incubation, if there are Rhizopus colonies or no. You have to keep them incubated, if no such colonies. As colonies are not formed after 3 days incubation, but after 4 or 5 days in some cases, you have to keep them incubated after 3 days incubation	
G) Staph. aureus			
1	What kind of sample is applicable?	Applicable in environmental test, especially in sanitation checks of hands.	Further biochemical examination is necessary for the precise identification.
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H) Salmonella			
1	Can I differentiate salmonella or Citrobacter on a sheet of the RIDA Salmonella Count?	Very slight color may be recognized in Citrobacter species. because the generation of iron sulfide is suppressed in Citrobacter species, while apparent dark color in Salmonella.	Further biochemical examination is necessary for the precise identification.
2	No necessary in enrichment?	If you want to detect salmonella with the same sensitivity of the conventional method, you should enrich before application to the sheets.	
I) Discarding			
1	How can I discard the sheets after use?	Discard the sheets after sterilization, such as autoclaving, boiling with hot water, etc.	
J) Stamping			
1	Leakage of solution?	Keep the sheets still for more than 15 min, more than 30 min if cold, after 1 mL application of the sterilized saline. Then, you can use them in stamping tests.	Sterilize the stamping site with a clean cloth.
2	Localized colonies?	Localized colonies appear when you smear the site instead of stamping. Stamping is more recommendable than smearing. You should slightly rub the sheets with you fingers. Do not squeeze the sheets!	

＊Please feel free to ask any question!
＊Please read the instruction manual well before use.

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