Fera Science Ltd (Fera)
York Biotech Campus
York, UK
YO41 1LZ

Tel: +44 (0)1904 462100
info@fapas.com
fapas.com

Fapas® – Food Microbiology Distribution 300
02 September 2024

Safety information:

For details on safe handling of the enclosed samples please download the Fapas® – Food Microbiology Safety Data Sheet from the relevant link at: fapas.com/technical-documentation

Test Material(s) dispatched:

One or more test material(s), as appropriate to your order:

M299e25	Enumeration of Aciduric Bacteria / Yeast / Mould (equivalent to 100 ml Soft Drinks),
M300e01	Enumeration of Lactic Acid Bacteria (equivalent to 10 g Beef),
M300e02	Enumeration of *Listeria monocytogenes* (equivalent to 10 g Smoked Fish),
M300e06	Enumeration of *Clostridium* spp. / *Clostridium perfringens* / Sulphite Reducing Clostridia (equivalent to 10 g Beef),
M300e12	Enumeration of *Bacillus cereus* (equivalent to 10 g Cooked Rice),
M300e23	Enumeration of APC / Lactic Acid Bacteria (equivalent to 10 ml Fruit Juice),
M300e24	Enumeration of APC / Enterobacteriaceae / Coliforms / *Escherichia coli* (equivalent to 10 g Pet Food),
M300d02	Detection of *Listeria monocytogenes* / *Listeria* spp. (equivalent to 25 g Mixed Vegetables`, x2),
M300d071	Detection of *Salmonella* spp. (equivalent to 25 g Chicken, x2),
M300d072	Detection of *Salmonella* spp. (equivalent to 25 g Milk Powder, x2),
M300d11	Detection of *Escherichia coli* O157:H7 (equivalent to 25 g Cheese, x2).

PLEASE NOTE: Test materials were dispatched chilled **BUT, even if they are not cold upon receipt, they will still be suitable for analysis**.

Instructions:

1) Store the test material(s) at **2-8°C** until analysis:
2) Start the analysis between **02 September** and **11 September 2024.**
3) Follow the sample preparation instructions given below. PLEASE NOTE:
   - It is essential that you follow the sample preparation instructions given below because Fapas® cannot be held responsible for errors arising from failure to comply with those instructions.
   - The sample preparation instructions are also available for download (in English and Spanish) from the relevant link at: fapas.com/technical-documentation.
4) Having followed the sample preparation instructions given below, treat each test material as if it was a sample for routine analysis You may use any method of analysis you wish.
5) **For proficiency tests in enumeration you must**:
   - Report your results in **cfu/g** or **cfu/ml** as appropriate, do NOT report results in $\log_{10}$ cfu/g or $\log_{10}$ cfu/ml.

- You may enter your results in scientific notation but if you do then you MUST use the correct format, e.g. you should enter, 2000 as 2.0E+3, *not* as $2x10^3$ or enter 34000 as 3.4E+4, *not* as $3.4x10^4$.

PLEASE NOTE: It is essential that you submit your results as stipulated above because Fapas® cannot be held responsible for errors arising from failure to comply with those instructions.

6) **For proficiency tests in detection**:
   - Two test materials (A and B) have been sent for tests M300d02, M300d071, M300d072 and M300d11.
   - You MUST report a result for **BOTH** test materials.
   - It is possible for either, both or neither of the samples to contain the target organism.
   - Enter your results, as **detected/not detected in 25 g**.
   - Please note that the detection of *Listeria* spp. includes the detection of *L. monocytogenes*.
   - The *E. coli* O157 strain used is atoxigenic (does not produce a toxin). The proficiency test is not suitable for tests which rely on detection of the genes for VT1/VT2, (STX1/STX2) because they are absent, but it does have eae genes.
7) Instructions on how to enter your results and methods via the secure web page can be downloaded from the relevant link at: fapas.com/technical-documentation.
   - You may submit more than one set of results.
   - By default, the **first** set of results you enter are those that will be assessed in the report BUT you may instead choose any additional entries.
8) When you enter your results, comments and methods please ensure you:
   - Use English, as it is the default international language.
   - Use Western characters. Entries made in other characters will be captured as symbols that are not readable.

Please ensure you submit your data no later than:

closing date 23 September 2024

You are reminded that the ability to report results in the specified units and within the given time scale are part of the proficiency test.

Please note that collusion between participants is contrary to professional scientific conduct and, as indicated in our Protocols (available at: fapas.com/technical-documentation), is strongly discouraged.

In October 2024 a statistical report on the performance of participating laboratories will be published on our secure web site. This report will be confidential and will reveal only the number assigned to your laboratory. It will not list the identities of participants.

If you have any problems please contact Fapas® immediately, email: info@fapas.com, tel: +44 (0)1904 462100.

Stilian Hristov

Proficiency Test Co-ordinator
On behalf of Fapas®

Fapas® – Food Microbiology

INSTRUCTIONS FOR THE PREPARATION OF SAMPLES

Notes

Find the Product Matrix Code appropriate to your sample by logging in at fapas.com and clicking 'Go to Results' then looking at the second half of the Product Code.

Prepare your sample according to Sample Preparation Procedure (A to J) shown for that Product Matrix Code.

Product Matrix Code	Sample Preparation Procedure
AFE1	B
CCP22	A
CCP28 Flour (detection of E. coli 0157:H7)	B
CCP28 Flour (enumeration of Yeasts and Moulds)	C
CON2	C
CON3	C
DRN17	F
DRN29	I
DRN41	J
DRY7	G
DRY14	B
DRY18	H
EGG3	A
INF10	C
MRP2	A
MRP14	A
MRP35	A
MRP47	A
NUT12	C
NUT30	C
PFO9	A
PRO40	A
SEA11	A
SEA20	A
SEA28	A
SPI11	C
SPI17	C
UNF11	D
UNF12	E
VEG47	A
VEG61	A
VEG71	A
VEG88	A

INSTRUCTIONS FOR THE PREPARATION OF SAMPLES

Procedure A

Beef, Chicken, Meat, Fish, Herbs (parsley), Egg, Salad, Rice, Mixed vegetables, Sprouting Seeds and Lettuce, Prawns, Pet Food and Ready To Eat (RTE) Meal

These samples require a **rehydration** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

For each sample for an **ENUMERATION test:** add 10 ml (+/- 0.2 ml) buffered peptone water at room temperature.
Important Note: this gives you a sample which is equivalent to 10 g of a routine sample.

For each sample for a **DETECTION test EXCEPT for *Vibrio parahaemolyticus*:** add 20 ml (+/- 0.2 ml) buffered peptone water at room temperature.
Important Note: this gives you a sample which is equivalent to 25 g of a routine sample.

For each sample for a **DETECTION test ONLY for *Vibrio parahaemolyticus:*** add 20 ml (+/- 0.2 ml) **ASPW** (Alkaline Saline Peptone Water) or equivalent at room temperature.
Important Note: this gives you a sample which is equivalent to 25 g of a routine sample.

Then:

- Gently invert the sample a few times to aid rehydration.
- Leave the sample to stand at room temperature for 30 minutes (+/- 2minutes).

The sample is now ready to test using your usual procedure.

Procedure B

Flour (detection of *E. coli* 0157:H7), Milk Powder and Animal Feed Test Materials

These samples require a **resuscitation** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

Add the sample to the blender / homogeniser bag.

For each sample for an **ENUMERATION test:** add 90 ml (+/- 2 ml) of your usual diluent at room temperature, rinsing the sample container with part of the diluent.
Important Note: this makes a 1/10 dilution.

For each sample for a **DETECTION test**: add 225 ml (+/- 5 ml) of your usual pre-enrichment / enrichment broth at room temperature, rinsing the sample container with part of the broth.
Important Note: this makes a 1/10 dilution.

Then:

- Leave the sample to stand at room temperature for 30 minutes (+/- 2 minutes).

The sample is now ready to test using your usual procedure.

Procedure C

Section 1 - Flour (enumeration of Yeasts and Moulds), Ground Pepper, Chocolate, Chocolate Powder, Spices, Nuts and Infant Formula

These samples can be analysed without any special preparation.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

For each sample for an **ENUMERATION test** OR a **DETECTION test.**

The sample is ready to test using your usual procedure.

Section 2 - Detection of Enterobacteriaceae, Coliforms and *Escherichia coli* in Infant Formula

1) Carefully remove the crimp cap of the small glass vial and discard it. Then aseptically remove the rubber bung and discard it.

2) Reconstitute each of the test materials by adding 1 ml of BPW to the glass vial and allow to rehydrate for 2 minutes.

3) Transfer the cocktail from the small glass vial into 90ml BPW, rinse the glass vial with the 90ml BPW to make sure all the solution is extracted.

4) Amalgamate the 10g infant formula from the plastic container and the 90ml BPW into a stomacher bag and stomach for 2 minutes.

The sample is ready to test using your usual procedure.

Procedure D

Swab (Sponge) for Detection and Enumeration

These samples require a **rehydration** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

Add 10 ml (+/- 0.2 ml) buffered peptone water at room temperature directly to the sponge in the container.

Then:

- Leave the sample to stand at room temperature for 30 minutes (+/- 2 minutes).

For the **ENUMERATION** test**:** Transfer the sample into a sterile stomacher bag with 90ml of non-selective pre-enrichment broth. You can use sterile forceps if necessary. Rinse the container and homogenise the sample using the stomacher. Please consider this as your first dilution.

The sample is now ready to test using your usual procedure.

Procedure E

Swab (Cotton)

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

Add 10 ml (+/- 0.2 ml) buffered peptone water at room temperature to the sample and vortex thoroughly for 30 seconds.

Then:

- Leave the sample to stand at room temperature for 30 minutes (+/- 2 minutes) to rehydrate.
- Vortex the sample for 10 seconds.

The sample is now ready to test using your usual procedure.

Procedure F

Fruit Juice

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the vial thoroughly.

Add 1ml (from a 10ml (+/- 0.2 ml) aliquot) of buffered peptone water at room temperature to the sample vial.

Then:

- Leave the sample to stand at room temperature for 1 minute (+/- 10 seconds).
- Carefully rinse the vial contents twice using a pastette (Pasteur pipette).
- Transfer the full contents from the vial into the 10ml buffered peptone water (from which the initial 1ml amount was taken) to give a final volume of 10ml (+/- 0.2 ml).

The sample is now ready to test using your usual procedure.

Procedure G

Cheese

These samples require a **rehydration** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

For each sample for an **ENUMERATION test:** add 10 ml (+/- 0.2 ml) buffered peptone water at room temperature.
Important Note: this gives you a sample which is equivalent to 10 g of a routine sample.

For each sample for a **DETECTION test** add 20 ml (+/- 0.2 ml) buffered peptone water at room temperature.
Important Note: this gives you a sample which is equivalent to 25 g of a routine sample.

Then:

- Gently invert the sample a few times to aid rehydration.
- Leave the sample to stand at room temperature for 30 minutes (+/- 2minutes).

The sample is now ready to test using your usual procedure.

Procedure H

Soft Cheese (detection of *Listeria monocytogenes*)

These samples can be analysed without any special preparation.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

For each sample for an **ENUMERATION test** OR a **DETECTION test.**

The sample is ready to test using your usual procedure.

Procedure I

Soft Drink

These samples require a **rehydration** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the container thoroughly.

Add 1ml from the 100ml simulated soft drink provided into the vial. Please ensure that the diluent used is at room temperature.

Then:

- Leave the sample to stand at room temperature for 1 minute (± 10 seconds).
- Carefully rinse the vial contents twice with the simulated soft drink using a pastette.
- Transfer the full contents from the vial into the 100ml simulated soft drink from which the initial volume of 1ml was taken.
- Gently mix the prepared sample by inversion

The sample is now ready to test using your usual procedure.

Procedure J

Water (Bottled)

These samples require a **rehydration** stage before you start the analysis.

TREAT EACH SAMPLE AS A WHOLE – DO NOT SUB-SAMPLE and ensure you rinse the containers thoroughly. Do NOT combine the test materials

Carefully remove the crimp cap and discard it. Aseptically remove the rubber bung and discard it. Reconstitute each of the test materials by adding 1 ml of sterile deionised/distilled water to the glass vial.

Then:

- Leave the sample to stand at room temperature for 2 minute (± 10 seconds).
- Dilute the resulting suspension to a final volume of 1000 ml ± 20 ml using your own sterile deionised / distilled water.
- Rinse the glass vial 2-3 times during this process using your sterile deionised / distilled water to ensure that all of the inoculum is added to the final 1000 ml ± 20 ml volume.
- Gently mix the prepared sample by inversion.

The sample is now ready to test using your usual procedure.