Fapas® – Food Microbiology

INSTRUCTIONS FOR THE PREPARATION OF SAMPLES

Fapas® Food Microbiology Scheme test materials require preparation by participants before analysis. To identify which procedure applies to your test material, you must determine the correct Product Matrix Code for your sample.

To do this, log in at fapas.com, select *'Go to Results'*, and locate the Product Code. Use the second half of the Product Code to identify the appropriate Product Matrix Code.

Prepare the sample using the Sample Preparation Procedure (A–L) specified for the relevant Product Matrix Code in the table on the next page.

Please note:

Microbiology test materials are usually freeze-dried to ensure stability under transportation conditions. Some matrices, such as milk powder, may no longer be a free-flowing powder after the freeze-drying process. This is normal and does not compromise the nature of the test material, especially if the reconstitution instructions are followed. In addition, some discolouration of milk powder can occur after the freeze-drying process, due to varying levels of fat in the originating milk. These test materials are still fit-for-purpose for the proficiency test.

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 Chocolate (Detection of Salmonella in 375g) ONLY	K
CON2	C
CON3	C
DRN17	F
DRN29	I
DRN41	J
DRY7	G
DRY14	B
DRY18	H
EGG3	A
FRU95	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 Swab (enumeration of Coagulase Positive Staphylococci only)	L
UNF11 Swab (DO NOT USE for Coagulase Positive Staphylococci)	D
UNF12	E
UNF16	B
VEG47	A
VEG61	A
VEG71	A
VEG88	A

Procedure A

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

These samples require a **reconstitution** 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 **EXCEPT for Enterobacteriaceae in 25 g Dried Egg:**

- 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 an **ENUMERATION** test **ONLY for Enterobacteriaceae in 25 g Dried Egg:**

- 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 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.

For all test materials

- Gently invert the sample a few times to aid reconstitution.
- 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, Animal Feed and Overshoe 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.

- 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) Resuscitation 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.

Important Note: this makes a 1/10 dilution.

The sample is ready to test using your usual procedure.

Procedure D

Swab (Sponge) for Detection and Enumeration (NOT including Coagulase Positive Staphylococci 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.

- Leave the sample to stand at room temperature for 30 minutes (+/- 2 minutes).
- Important Note: This is your neat sample

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. .

- Important Note: this makes a 1/10 dilution.

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

Procedure E

Swab (Cotton)

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 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.
- Important Note: This gives you a neat sample.

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

Procedure F

Fruit Juice

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

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).
- Important Note: This gives you a neat sample.
- The sample is now ready to test using your usual procedure.

Procedure G

Cheese

These samples require a **reconstitution** 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 **reconstitution** 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
- Important Note: This gives you a neat sample.

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

Procedure J

Water (Bottled)

These samples require a **reconstitution** 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.

Procedure K

375g Chocolate

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

This proficiency test is a combination of 15 test materials (equivalent to 25g each) for the detection of *Salmonella* spp. in a total 375g.

Each test material must be treated as 25g (**please do not weigh the samples and do not test the samples individually).**

Ensure that the entire sample from each test material pot is transferred, and the container is thoroughly rinsed.

3375 ml prewarmed enrichment broth must be used in total for the 15 test materials.

All 15 samples can be pooled in a bag with **3375 ml** prewarmed enrichment broth.

If smaller pools are necessary, it is important that **225ml** of prewarmed enrichment broth is added per test material.

The sample is ready to test using your usual procedure.

The result must be reported as detected or not detected in 375g

Procedure L

Swab (Sponge) and separate inoculum vial – applies to Coagulase Positive Staphylococci enumeration ONLY

The test material comprises of an inoculum vial and separate swab vial

The inoculum vial requires a **reconstitution** stage before you start the analysis.

Swab vial

- Aseptically remove the swab from the vial (you can use sterile forceps if necessary).and place in a sterile stomacher bag.

inoculum vial

- Remove the crimp cap from the inoculum vial and aseptically remove the bung.
- Reconstitute vial using 1ml portion of 10ml Buffered Peptone Water.
- Leave at room temperature for 1 minute ± 10 seconds.
- Rinse the vial twice and transfer the full contents of the vial back into the 10ml BPW.
- Transfer the sample into the sterile stomacher bag containing the swab 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.
- Important Note: this makes a 1/10 dilution