

## ***Instructions for Handling Test Materials and Recording Results***

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### **Receipt and Storage**

- On receipt of the test material, record the date and store refrigerated at 2-8°C until ready to test.
- The test material should be analysed in accordance with the deadlines shown on the website:  
<https://portal.lgcstandards.com>

### **Sample Details**

- The test materials represent 'real' food or dairy samples, which may or may not contain the target organism(s), at a range of inoculum levels. Test materials may also contain background flora.
- Please consider all dilution factors when calculating the level of the target organisms in the original sample, where relevant guidance is given in the instructions.
- The test materials should be treated in the same way as routine samples and should therefore be tested using your routine laboratory equipment, reagents and methods.
- Where the instruction mentions a **resuscitate** step, the lyophilised test material should be left at room temperature for between 60 to 90 minutes before proceeding with the test.
- Do not sub-divide samples prior to dilution unless stated in the instructions.
- Please treat the sample as contaminated material and use aseptic techniques throughout the test procedure.
- Please ensure samples are mixed well at all stages before proceeding with testing.
- Reconstitute the test material according to the appropriate instructions for each sample type as described below;

### **Format: 10g matrix only (enumeration)**

**Samples: 03, 05, 08, 09, 10, 11, 15, 16, 17, 18, 20, 23, 24 (except 24H and 24SP), 25, 26**

1. Prepare 90ml of suitable microbiological diluent and add to the 10g sample.
2. Mix the diluted test material thoroughly and allow to **resuscitate** before testing.
3. The 10g sample should be treated as neat for dilution calculations.

### **Format: 25g matrix (detection)**

**Sample: 04**

1. Decide whether you wish to test the sample as a 25g portion or a 10g portion.
2. If you wish to test the sample as a 10g portion aseptically weigh a 10g portion from the matrix provided.
3. Prepare 225ml or 90ml of your chosen enrichment broth depending on the sample size you are testing.
4. Add the enrichment broth to the 10g or 25g sample.
5. Mix the diluted test material thoroughly under aseptic conditions and leave to **resuscitate** before testing.

### **Format 25g matrix only (detection)**

**Samples: 06 (except 06C and 06S), 07 (except 07C and 07H), 12, 13, 14, 22**

1. Prepare 225ml of your chosen enrichment broth and add to the 25g sample.
2. Mix the diluted test material thoroughly and allow to **resuscitate** before testing.

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### **Format: Vial + 10g matrix (enumeration)**

**Samples: 24H, 24SP, 29, 41, 45**

1. Prepare 90ml of suitable microbiological diluent.
2. From this volume take 10ml and add it to the vial.
3. Replace the vial stopper and shake gently to dissolve. Allow to **resuscitate** before proceeding.
4. Reconstitute the matrix with the remaining amount of diluent prepared in step 1.
5. After the resuscitation period add the vial contents prepared in step 3 to the matrix preparation from step 4, back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial.
6. The 10g sample should be treated as neat for dilution calculations.

### **Format: Vial + 25g matrix (detection)**

**Samples: 06C, 06S, 07C, 07H, 28, 30, 31, 42**

1. Prepare 225ml of your chosen enrichment broth.
2. From this volume take 10ml and add it to the vial.
3. Replace the vial stopper and shake to dissolve, then leave the test material to **resuscitate**.
4. Reconstitute the matrix with the remaining amount of diluent prepared in step 1.
5. After the resuscitation period add the vial contents prepared in step 3 to the matrix preparation from step 4 back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial.

### **Format: Vial only-no matrix (enumeration)**

**Sample: 27, 32**

1. Prepare 10ml of suitable microbiological diluent.
2. Add the 10ml of diluent prepared in step 1 to the vial.
3. Replace the stopper and shake to dissolve, then leave the test material to **resuscitate**.
4. Immediately before testing, mix the resuscitated sample thoroughly.
5. The resuscitated sample in 10ml diluent should be treated as the neat sample **i.e. do not take the initial 10ml dilution into account when calculating results.**

### **Format: Vial only-no matrix (detection)**

**Sample: 21**

1. Prepare 225ml of your chosen enrichment broth.
2. From this volume take 10ml and add it to the vial.
3. Replace the stopper and shake to dissolve, then leave the test material to **resuscitate**.
4. Add the vial contents to the remaining enrichment broth prepared in step 1, back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial.

### **Format: Vial only-no matrix (identification)**

**Sample: 33, 34**

1. Prepare 9ml of suitable microbiological diluent.
2. Add the 9ml of diluent prepared in step 1 to the vial.
3. Replace the stopper and shake to dissolve and leave the test material to **resuscitate**.
4. Immediately before testing, mix the resuscitated sample thoroughly.
5. Streak onto non-selective media and incubate at mesophilic temperature.
6. Using your microbiological knowledge and following standard microbiology tests determine the identification and/or serology of the organism contained in the test material.

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**Format: Paper Exercise**

**Sample: 35**

1. Please refer to instruction sheet provided.

**Format: 2x Vial + minimum 20g matrix (enumeration)**

**Sample: 36**

1. Vials are labelled A and B. For each vial, weigh out 10g of matrix and prepare 90ml of suitable microbiological diluent.
2. For each vial, take 10ml of the 90ml diluent and add it to the vial after aseptically removing cap and rubber stopper.
3. Replace the stopper and shake to dissolve, and leave the test material to **resuscitate**.
4. Reconstitute the 10g matrix with the remaining amount of diluent prepared in step 1.
5. After the resuscitation period add the vial contents prepared in step 2 to the matrix preparation from step 4 back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial. The test material is now ready to test.
6. Remember to take the initial dilution factor into account when calculating results.

**Note:** The two vials will have different contents, please handle separately at all times and use clear labelling at all stages of processing to avoid mixing up vial A and vial B.

**Format: Vial + minimum 20g matrix (detection)**

**Sample: 38**

1. Prepare 10ml of suitable microbiological diluent.
2. Aseptically remove the cap and rubber stopper from the vial and reconstitute the freeze-dried test material by adding the 10ml of diluent prepared in step 1.
3. Replace the stopper and shake to dissolve and leave the test material to **resuscitate**.
4. For each analyte under test, aseptically weigh out 10g of matrix and prepare 90ml of suitable microbiological diluent.
5. For each analyte, add the 90ml diluent to the 10g matrix.
6. After the resuscitation period add 1ml of the vial contents prepared in step 2 to each of the reconstituted matrix preparations and mix thoroughly. The test material is now ready to test.

**Format: 2x Vial + minimum 200g matrix (detection)**

**Sample: 37**

1. Vials are labelled A and B. For each vial, prepare 10ml of a suitable microbiological diluent e.g. maximum recovery diluent (MRD).
2. For each vial, add the 10ml of diluent prepared in step 1. Shake to dissolve and leave to **resuscitate**.
3. For each vial, and each analyte under test, aseptically weigh out 25g of matrix.
4. Prepare 225ml of your chosen enrichment broth for each analyte under test, noting that different enrichment broths will be required for different analytes.
5. Add 225ml enrichment broth to the 25g portion prepared in step 3.
6. After the resuscitation period add 1ml of the vial contents prepared in step 2 to the matrix preparation from step 5.

**Note:** The two vials will have different contents, please handle separately at all times and use clear labelling at all stages of processing to avoid mixing up vial A and vial B.

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#### **Format: 10g matrix only**

##### **Sample: 39**

1. Prepare 90ml of suitable microbiological diluent and add to the 10g sample of skimmed milk powder.
2. Mix the diluted test material thoroughly under aseptic conditions and leave the test material to **resuscitate**.
3. The resuscitated liquid sample should be treated as the neat sample for testing **i.e. do not take the initial dilution into account when calculating results.**

#### **Format: Vial + minimum 375g matrix (detection)**

##### **Sample: 40, 43**

1. Prepare the appropriate volume of your chosen enrichment broth.
2. From this volume take 10ml and add it to the vial after aseptically removing cap and rubber stopper.
3. Replace the vial stopper and shake to dissolve and leave the test material to **resuscitate**.
4. Weigh out 375g of matrix and reconstitute with the remaining amount of diluent prepared in step 1.
5. After the resuscitation period add the vial contents prepared in step 2 to the matrix preparation from step 4 back-washing two or three times to ensure all the freeze-dried test material is recovered from the vial. Mix the diluted test material thoroughly under aseptic conditions.

#### **Format: Vial + minimum 100g matrix (detection)**

##### **Sample: 44**

1. Prepare 10ml of a suitable microbiological diluent e.g. maximum recovery diluent (MRD).
2. Add the 10ml of diluent prepared in step 1 to the vial. Shake to dissolve and leave to **resuscitate**.
3. For each analyte under test, aseptically weigh out 25g of matrix
4. Prepare 225ml of your chosen enrichment broth for each analyte under test, noting that different enrichment broths will be required for different analytes.
5. Add 225ml enrichment broth to the 25g portion prepared in step 3.
6. After the resuscitation period add 1ml of the vial contents prepared in step 2 to the matrix preparation from step 5.

#### **Recording Results**

- All results should be submitted using PORTAL
- Please go to <https://portal.lgcstandards.com>
- Login using your Lab ID, username and password.
- A PORTAL user guide can be downloaded from the help section.

If you need any help at all please do not hesitate to contact our support team using the details below or your local LGC representative.

Tel: +44(0)161 762 2500

Email: [ptcustomerservices@lgcgroup.com](mailto:ptcustomerservices@lgcgroup.com)

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### **Precautions**

- Test materials contain viable micro-organisms and are supplied on the understanding that the purchaser has suitably competent and qualified personnel to handle them safely. Test materials must only be opened in a laboratory by qualified personnel.
- Refer to the Safety Data Sheet for information on the safe handling and disposal of the test materials.