

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

### **Receipt and Storage**

- On receipt of the test material, record the date and store refrigerated at 2-8°C until ready to test, with the exception of Sample 606 which should be stored at room temperature.
- The test material should be analysed in accordance with the deadlines shown on the website: <https://www.lgcpt.com/portal>.

### **Microbiology samples**

#### **Sample Details**

- The test material represents a 'real' gelatine sample, which may or may not contain the target organism(s), at a range of inoculum levels. Background flora may also be present.

#### **Resuscitation**

1. Test the entire sample; do not sub-divide prior to dilution.
2. Dilute your test material according to its type as below, then proceed to step 3:

For **enumeration tests**, dilute the sample initially by 1 in 10 using your chosen diluent i.e. by adding 90ml of diluent to the 10g sample.

For **presence/absence** tests, dilute the sample with your chosen diluent or enrichment broth, according to your usual laboratory procedure.

3. Mix the test material thoroughly under aseptic conditions.
4. Leave the test material to resuscitate at room temperature for a minimum of 60 minutes, but no longer than 90 minutes.

#### **Testing**

- Immediately before testing, mix the resuscitated sample thoroughly and then test for the target organism(s) using your routine laboratory methods.

### **Chemistry samples**

#### **Determination of ash**

1. Weigh approximately 5.0 g of gelatine to 0.001 g in a suitable crucible that has previously been ignited, cooled and weighed.
2. Add 1.5 to 2.0 g of paraffin to avoid loss due to swelling.
3. Heat, gently at first on an electric hot plate or in a muffle furnace, until the substance is thoroughly charred.
4. Finish ashing in a muffle furnace at 550°C for 15 to 20 hours.
5. Cool the sample in a desiccator.
6. Weigh the sample and calculate the percentage of residue

The ash content, expressed as % ash, is equal to:

$$\% \text{ ash} = [\text{weight of ash}] / [\text{weight of sample}] \times 100\%$$

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### **Determination of gel strength (Bloom value)**

1. Apparatus: Texture analyser or gelometer with a cylindrical piston  $12.7 \pm 0.1$  mm in diameter with a plane pressure surface with a sharp bottom edge, or a bottle  $59 \pm 1$  mm in internal diameter and 85 mm high.
2. Adjust the apparatus according to the manufacturer's manual. Settings are: distance 4 mm, test speed 0.5 mm/s.
3. Method Perform the test in duplicate. Place 7.5 g of the substance to be tested in each bottle. Add 105 ml of water R, place a watch-glass over each bottle and allow to stand for 1-4 h. Heat in a water-bath at  $65 \pm 2$  °C for 15 min. While heating, gently stir with a glass rod. Ensure that the solution is uniform and that any condensed water on the inner walls of the bottle is incorporated. Allow to cool at room temperature for 15 min and transfer the bottles to a thermostatically controlled bath at  $10.0 \pm 0.1$  °C, and fitted with a device to ensure that the platform on which the bottles stand is perfectly horizontal. Close the bottles with a rubber stopper and allow to stand for  $17 \pm 1$  h. Remove the sample bottles from the bath and quickly wipe the water from the exterior of the bottle. Centre consecutively the 2 bottles on the platform of the apparatus so that the plunger contacts the sample as nearly at its midpoint as possible and start the measurement.
4. Report the result as the average of the 2 measurements.

### **Determination of isoelectric point**

1. Prepare a 5% gelatine solution and completely deionise by treating with ion exchange resins.
2. Measure pH of the solution obtained at 30°C, the reading corresponds to the isoelectric point of gelatine.

### **Determination of moisture**

1. Wash the evaporating dish very carefully in hot water.
2. Place the dish in the drying oven at 105°C for at least one hour.
3. Cool dish in the desiccator until room temperature is reached.
4. Weigh approximately 5.0 g of gelatine to the nearest milligram and note the weight of the test sample ( $m_0$ ) and the weight of the sample together with the evaporating dish ( $m_1$ ).
5. Place the evaporating dish containing the sample in the drying oven at  $105 \pm 2$ °C for 16 to 18 hours.
6. Cool the dish in the desiccator until room temperature is reached and weigh to the nearest milligram ( $m_2$ ), weigh and calculate the percentage of residue.

The moisture content, expressed as a percentage by weight, is equal to:

$$\% \text{ Moisture} = [(m_1 - m_2) / m_0] \times 100\%$$

where:  $m_0$  is the weight in grams of the test sample,  $m_1$  is the weight in grams of the test sample and the evaporating dish, before drying  $m_2$  is the weight in grams of the test sample and the evaporating dish, after drying.

### **Determination of pH**

1. Dissolve 1.00 g gelatine in carbon dioxide-free water R at about 55 °C, dilute to 100 ml with the same solvent and keep the solution at this temperature to carry out the tests.

*Carbon dioxide-free water R:* Water that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage.

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### **Determination of viscosity**

1. Weigh  $7.50 \pm 0.01$  g gelatine into a bloom jar or 150 mL beaker.
2. Add  $105.0 \pm 0.2$  g deionized water, stirring often to suspend all gelatine particles.
3. Cover and let stand 1 – 3 hours at room temperature
4. Dissolve the sample in a 65°C water bath for 10 – 15 minutes, stirring or swirling as required.
5. When the temperature of the solution reaches 61°C, and the sample is completely dissolved and thoroughly mixed, transfer the solution to the viscosity pipette and proceed with the viscosity determination.
6. Using a finger of the free hand cover the capillary end of the pipette and pour enough solution into the pipette to bring the level approximately 1 cm above the upper mark.
7. Place the thermometer inside the pipette and slowly raise and lower it until a constant temperature of  $60.00 \pm 0.05^\circ\text{C}$  is maintained.
8. Remove the thermometer from the pipette.
9. Read and record the time required for 100 mL of solution to pass through the capillary tube of the pipette by draining the gelatine solution and starting the stopwatch as soon as the meniscus of the liquid hits the top line of the pipette. Stop the stopwatch when the meniscus hits the lower line of the pipette.
10. Record the time obtained to the nearest tenth of a second; this value is the efflux time.

### Calculation of the Viscosity:

The viscosity (to the nearest millipoise) at 60°C of any sample with efflux time  $t$  (in seconds) may be calculated from the following equation:

$$V = (At - B/t) \times d$$

A, B = A and B pipette constants

$t$  = efflux time, in seconds

$d$  = solution density (for a 6.67% gelatine solution at 60°C  $d = 1.001$ )

### **Recording Results**

- All results should be submitted using PORTAL
- Please go to <https://www.lgcpt.com/portal>
- 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: [support@lgcgroup.com](mailto:support@lgcgroup.com)

### **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.