13 Microbiological Methods

Preparation of samples (INEC TC)

1 Sampling and handling of samples

1.1 *Sampling plans:* Measure 200 g samples from a minimum of five containers or bags representing the shipment or lot to examined or use another suitable sampling plan

1.2 *Instructions for sampling:* Use aseptic technique; sterilise the sampling equipment after use. A trier may be used to sample most of the powdery galactomannan gums. Devices such as clean spoons, scoops or similar implements may also be used.

1.3 *Storage of samples until examination:* Place the samples in sterile plastic bags or wide-neck glass bottles, label clearly and forward to the microbiological laboratory without delay

1.4 *Storage temperature:* Not more than 20°C

2 Preparation of samples

Examine all five sample units separately, if possible. Combine only as a last resort when lack of facilities or time prevents the recommended amount of testing. Retain the individual samples for additional testing when indicated.

2.1 *Weigh-In quantity:* Aseptically weigh 1.00 g of the well mixed representative sample into a sterile weighing-boat.

2.2 *Diluent:* Phosphate buffer

2.2.1 *Ingredients of diluents:* Butterfield's buffered phosphate diluent:

Stock Solution: 34.0 g Mono-potassium-hydrogen-phosphate, 500.0 ml distilled water. Adjust to pH 7,2 with 175 ml N-sodium-hydroxide solution, dilute to one litre and store.

Diluent: Dilute 1.25 ml of stock solution to one litre with distilled water. Prepare dilution blanks in suitable containers. Sterilise at 121°C for 15min.

2.2.2 *Amount of diluent:* 199 ml ±3 ml

2.3 Blending and/or homogenisation

Caution: Thickening-agents like galactomannans may form lumps during the addition of diluent. In the first instance pour aseptically 199±3ml of diluent into a sterile 250 or 300 ml wide-mouth polypropylene or glass bottle with sterile stopper. Subsequently add carefully the weighed-in sample accompanied by simultaneous moderate shaking by hand. The formation of lumps in the dilution must be consistently avoided, otherwise the preparation of sample dilution must be repeated.

After sealing the bottle with the stopper, shake the dilution vigorously to distribute any agglomerates of thickening-agent

Shake the prepared dilutions for 30 min at about 200 strokes per minute with a mechanical shaker. Inoculate appropriate media within 30 min.

2.4 Dilutions

If necessary, stock dilution (5×10^{-3}) is rediluted to 10^{-3} by mixing one part of sample dilution with four parts of diluent.