Method Comparison

Inhomogeneous dilution poses a potential risk to food quality & safety

The International Standard ISO 6887-1: 1999 defines a mixing time of preferably 5 to 10 seconds for serial dilutions in test tubes when using a mechanical stirrer (Vortexer).
In tests with diluted ultra heat treated (UHT) milk, turbidity measurements confirm that mixing times below 5 seconds result in inhomogeneous dilutions with large variations between the individual samples. The measured sample concentrations were found to be typically below the reference value. As a consequence bacterial counts on food samples, which were mixed for less than 5 seconds, gave low results and therefore pose a potential risk to labs making bad decisions in terms of food quality and food safety.
By contrast, using the Inlabtec Serial Diluter with the same samples, accurate dilutions with minimal deviations were achieved in less than 4 seconds. The Inlabtec Serial Diluter consequently offers a more reliable and accurate sample mixing protocol which can lead to improved food quality and safety. By removing the need for technicians to manually handle and vortex large numbers of test tubes – the Inlabtec Serial Diluter also eliminates the risk of repetitive strain injury (RSI).

Introduction

Serial dilution protocol dictates that samples of 1 ml must be homogeneously mixed with 9 ml of diluent. For laboratories following the ISO 6887-1: 1999 standard for serial dilutions using test tubes a mixing time of preferably 5 to 10 seconds using a mechanical stirrer (Vortexer) is stated. In busy laboratories, there is a risk that mixing time may sometimes be reduced to less than five seconds to enhance productivity.
To determine the effect of mixing time on the quality of the bacterial count food testing - a series of turbidity measurements were made on samples mixed for varying times. Turbidity is defined as the reduction in transparency of a liquid caused by the presence of undissolved components, such as particles, vesicles, microorganisms, etc. Therefore if samples taken from the same dilution step, but with differing mixing times, produce varying turbidity measurements this indicates that the amount of undissolved components in the sample is not consistent and therefore, the liquid was not homogeneously diluted. Turbidity can be easily and precisely determined by measuring the attenuation of light (optical density).

Method

Ultra-high temperature (UHT) processed milk was chosen as a model food sample to determine the homogeneity of dilutions. UHT milk is readily available and offers a stable fat-in-water emulsion in which the undissolved fat is distributed in fat globules within the liquid milk. By homogenizing UHT milk the fat globules are standardized to a diameter of about 1 micron, which corresponds to the diameter of most bacteria. The requirements for the mixing process for homogeneous dilution of UHT milk are therefore similar to bacteria contaminated food samples.
Using the pre-filled test tube standard method, twelve 1:10 dilutions of UHT milk with water employing mixing times of 2, 3, 4, 5, and greater than 6 seconds were created (a total of 60 dilutions). For the mixing time of greater than 6 seconds, 1 ml of UHT milk was purged from the pipette tip by repeated dispensing and aspiration of water and the sample then mixed with a vortexer for 6 - 8 seconds. After dilution and mixing 1 ml samples were taken and the optical density (OD) measured at 820 nm using a LKB Novaspec II photometer. The mean and the standard deviation of the 12 measurements at each mixing time were determined. The mean optical density of the homogeneous samples created from greater than 6 seconds mixing time was defined as the reference 100% relative optical density value.

The same series of dilutions using 1 ml of UHT milk were created using an Inlabtec Serial Diluter and the optical density measured of these samples again measured at 820 nm.

Results

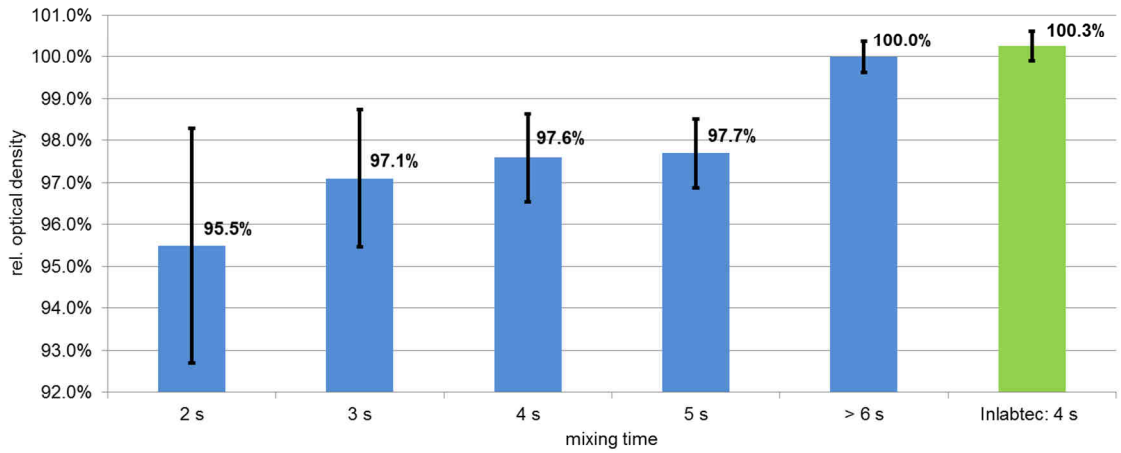

Fig. 1: Sample homogeneity as a function of mixing time measured by optical density.

Homogeneous dilutions were obtained with the test tube method after greater than 6 seconds mixing time, therefore the relative optical density of these samples was designated as 100% (Fig. 1). With mixing times of less than 5 seconds using the standard test tube method yielded increasingly low optical density values with greater standard deviation, as mixing time decreased. Using the Inlabtec Serial Diluter, with just 4 seconds mixing time, optical density and standard deviation values were achieved corresponding to the 'homogeneous sample' values from the classical technique with a mixing time greater than 6 seconds. The difference between the mean values of the Inlabtec Serial Diluter and the classical method (greater than 6 seconds mixing time) of 0.3 % is not significant and most probably the result of differences in volumes. The standard deviations measured for both dilution methods was only 0.4 %.

Significantly lower optical densities and therefore correspondingly lower concentrations of milk fat globules were measured in samples created by the classical test tube method after mixing times of from 2 to 5 seconds (verified by t-test). The mean optical density is around 97 % (Fig. 1). All the measured optical densities were significantly below 100 % relative optical density. Using the classical test tube technique samples mixed for 5 second demonstrated a doubling of standard deviation to 0.8 %. For a mixing time of 2 seconds the increased sample inhomogeneity of classically prepared samples was demonstrated (standard deviation 2.8%, 95.5% relative optical density).

Discussion

The measurements confirm for the classical test tube method that mixing time greatly affects the quality of the test results. The determined effect of mixing time on the homogeneity of the diluted sample and the negative deviation from the reference value is based on the mixing process itself and can be explained as follows.

Using addition of the sample by pipette, the dispensed sample is injected into the lower part of the test tube, which for UHT milk samples is clearly visible by eye. Through the subsequent vortexing the added sample must spread from the bottom to the top of the test tube. Subsequent sampling after mixing takes place at the top of the liquid as for accurate sampling the pipette tip should be immersed for only 2 – 5 mm (good pipetting practice). A measured absorbance value of below 100% relative optical density means that the sample was not fully dispersed in the diluent. In such a situation, the selected mixing time was too short to achieve a homogeneous dilution and differences in concentration between the lower and upper zone in the test tube still exist.

Our measurements confirm the relevance of the ISO 6887-1: 1999 recommended mixing times of 5 to 10 seconds which should be strictly followed if using the test tube method in combination with a vortexer. Mixing times of less than 4 seconds may already produce deviations of up to - 7.5% per dilution step from the correct value and in the worst case errors of up to - 20.8 % are already possible after 3 dilution steps. Viability counts based on too short mixing times per dilution can result in artificially low bacterial counts and therefore are a potential source of error with respect to the quality assessment of food safety.

With the Inlabtec Serial diluter, the sample is homogeneously diluted within a fixed mixing time of 4 seconds (Fig. 1). The high speed at which the sample in the pipette tip is rinsed out by the diluent into the Serial Dilution Bag immediately generates an intensive vortexing. After the complete delivery of the liquid an automatic 1 second burst of sterile air produces additionally chaotic mixing ensuring a perfect dilution. This automatic process is achieved by pressing a single button and guarantees consistently highly accurate and precise analytical results. Also, the RSI risk inherent in the classical test tube technique is completely eliminated since the repetitive mechanical mixing and handling of the test tubes is not necessary anymore.

References

INTERNATIONAL STANDARD ISO 6887-1 Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions