

HUMAN BIOLOGY
Unit 3X Externally Marked Practical Assignment

HBI3X/TN

Teachers' Notes

CONFIDENTIAL

These notes should be read in conjunction with *Instructions for the Administration of the Externally Marked Practical Assignment* published on the AQA website.

The number of bacteria in milk

Candidates will investigate one method of assessing the suitability of milk for human consumption. The method is based on the colour changes of an indicator (resazurin) that occur as it is reduced. To help candidates, the investigation will focus on the relative number of bacteria in milk.

Task 1

Materials

In addition to access to general laboratory equipment, each candidate needs

- 50 cm³ of whole fresh milk and 50 cm³ of whole stale milk
- 5 cm³ 0.005% resazurin solution
- a large beaker (400 cm³ or 500 cm³) to use as a water bath
- a small beaker (100 cm³)
- thermometer capable of measuring over the range 10 °C to 30 °C
- 5 test tubes and a way of covering the tubes such as suitable sized rubber bungs, caps, foil, or cling film but *not* corks
- test tube rack
- timer
- Bunsen burner
- tripod and gauze
- method for lighting Bunsen burner
- 4 graduated pipettes or syringes capable of measuring up to 5 cm³
- marker pen.

Managing the investigation

Candidates should be provided with two whole pasteurised milk samples of different ages. One should be a sample of fresh milk and the other a sample of stale milk. Local circumstances will affect milk samples but the stale milk should enable a colour change to be seen within about 30 minutes. Centres can choose how to produce stale milk but in a trial run, eight-day-old milk (milk kept refrigerated for 8 days after purchase) was found to be suitable.

Although the actual assessment process uses the full range of colours, to save time, this investigation will use as its end-point when the resazurin has become pink.

In this investigation, teachers must not give candidates the following information.

- How to interpret colour changes.
- What variables to monitor or control.

One week before sitting Task 1 of the EMPA, teachers may give candidates the following information.

The investigation will be about methods of estimating the number of bacteria in milk.

There should be no further discussion of this topic.

Task 2

Materials

In addition to access to general laboratory equipment each candidate needs

- 100 cm³ of milk sample
- 20 cm³ 0.005 % resazurin solution
- 5 large beakers (400 cm³ or 500 cm³) to use as water baths or access to thermostatic water baths (candidates could share a water bath but there should be no other cooperation)
- thermometer or thermometers capable of measuring over the range 15 °C to 70 °C
- 15 test tubes and bungs (or alternative way of sealing as in Task 1)
- timer
- 2 graduated pipettes or syringes capable of measuring up to 5 cm³
- marker pen.

Managing the investigation

All candidates should be provided with a similar sample of pasteurised milk. This could be the same age as the sample of stale milk used in Task 1. In Task 2, the rate of colour change to pink should be found at five different temperatures determined by the centre but the lowest temperature used should not be below 20 °C. A range of 20 °C to 60 °C is suggested but not prescribed to allow for differences between local samples of milk. The centre will need to decide for itself, through an appropriate trial, what constitutes a suitable range.

For processing of data and plotting of the graph, results from the five temperatures are required. The choice of age of the milk sample and temperatures *should* allow the candidates to collect results at the five different temperatures within the session but, as a minimum, results *must* be possible at three temperatures. Where a candidate is unable to obtain results from all five temperatures, the teacher should provide supplementary data.

In this investigation, teachers must not give candidates the following information.

- How to interpret colour changes.
- The number of repeats required.
- What variables to monitor or control.