# The effects of transport on microbiological proficiency test samples



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# Introduction

One of the challenges for providers of proficiency testing (PT) schemes is to demonstrate that samples are sufficiently stable to ensure that they will not undergo any significant changes throughout the conduct of the proficiency testing scheme, including during storage and transport, as described in ISO/IEC 17043:2010. Although stability tests can be performed in-house, it is impossible to predict and reproduce all possible environmental conditions that samples could be exposed to during transport, including temperature, humidity and atmospheric pressure. Although measures can be taken by the PT scheme organiser, such as refridgerated transport, these can increase costs and are still no guarantee that all samples are exposed to the same conditions during transport.

LGC have been organising PT schemes for over 20 years and have participants located in over 150 countries around the world. One of the biggest and longest running schemes is the food microbiology scheme, QMS, which has over 2000 participants. In order to improve stability, all QMS samples are prepared using lyophilised microorganisms. This study therefore looks at the extensive data available from QMS to see if, and how, various transport factors affect participant results.

# Method

Data from QMS samples distributed between 2009 and 2014 was assessed for a number of microbiological examinations, including total aerobic mesophilic count (TAMC), and enumeration of *Staphylococcus aureus*, Enterobacteriaceae, *Escherichia coli, Bacillus cereus*, *Pseudomonas* species and lactic acid bacteria. Matrices included oatmeal and skimmed milk powder (SMP). Over 76,000 individual test results were included in the study.

The participant results were compared against date of analysis, average distance travelled (km) and average temperature (°C) of destination country in order to determine if there were any significant trends between participants' results and these conditions.

### **Effects of temperature**

Based on the country in which each participant was based, the average annual temperature was estimated using the website www.weatherbase.com/weather/countryall. Participant z scores were then plotted against the average temperature. The highest average temperatures were seen in Barbados, Ghana, United Arab Emirates, Oman, Thailand and Nigeria, whilst the lowest were seen in Canada, Finland and Greenland.

**Graph 3:** Effect of temperature on results for enumeration of *Escherichia coli* in SMP in all QMS round from 2009 to 2014.



In this case, temperature of destination country did not appear to affect participant results.

The average temperature of a country gives an estimate of the likely transport temperature during a PT round but does not take into account seasonal fluctuations. Results from Italy were therefore assessed separately according to the average monthly temperature.

Graph 4: Average monthly temperature in Italy



**Graph 6:** Effect of distance travelled (km) on results for the enumeration of *Escherichia coli in* SMP in QMS rounds from 2009 to 2014.



It would be expected that PT samples which have to travel the furthest distances would be exposed to a greater number of environmental factors which may affect the outcome of testing. However, for *Escherichia coli* in skimmed milk powder, plotting of almost 4000 individual results over 50 rounds do not show any indication that results are influenced by distance travelled.

The average z score of all participants for all microorganisms and rounds was therefore also plotted against upon distance travelled, and this showed no significant relationship.

### Graph 7: Average z score compared to distance travelled



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### Effects of analysis date

Firstly, the analysis dates reported by participants were compared to the dispatch date to see when each participant typically tests the PT sample.

**Graph 1:** Days after dispatch that each participant reported performing the analysis



The data shows that the typical analysis date was 8 days after the dispatch date, with 80% of participants receiving and testing the sample within 10 days of dispatch date, and 99% within 18 days of dispatch.

**Graph 2:** Individual z scores of participants compared to analysis date for the enumeration of *Pseudomonas* species in oatmeal in all QMS rounds from 2009 and 2014



This graph shows data from 897 results over 13 PT rounds with Pseudomonas levels ranging from 380 to 21,000 cfu/g. There was no significant difference between results tested 1 day after dispatch compared to 25 days after dispatch.

**Graph 5:** All participant results for Italy compared to average temperature during month of dispatch



This graph included over 12000 results from 40 different PT rounds and included a range of different microorganisms, matrices and levels. The result showed that climate during the month of distribution does not appear to have any significant effect on participant's individual performance scores.

## **Effects of distance**

Based on the country in which each participant was based, the average distance in kilometres (km) that the PT samples would travel from the UK was estimated using the website www.timeanddate.com/worldclock/ distance.

Participant z scores were then plotted against the average distance travelled. The samples which travelled the least distance were UK, Ireland, Belgium and France. The samples that travelled the furthest travelled around 17,000km to Australia.



### **Performance by country**

It would be expected that if transport affected samples significantly, then this would mean that partipant results may be affected by the country in which they are based. The average z score for all countries taking part in QMS between 2009 and 2014 was therefore calculated using data for all microorganisms, as well as for individual organisms.

Graph 8: Comparison of performance scores by country



It can be seen that countries that are quite different in terms of climate and distance from the UK obtain similar performance scores, for example, United Arab Emirates, Brazil, Romania and Ghana. Of all the countries assessed, all the average z scores were within the range of  $\pm 1$ .

# Discussion

When a participant obtains a poor result in a PT scheme, they often assume the problem is with the sample, especially if it has travelled a long distance in high temperatures. However, these studies do not indicate that performance is significantly affected by analysis date, temperature or distance travelled.

There are many reasons for a participant obtaining low z scores, other than stability of the PT sample during transport. Laboratories, who may receive a sample within one day and test immediately, can still obtain unsatisfactory z scores which are obviously unrelated to transport factors. Similarly it can often be seen where participants in the same country, or even in the same laboratory, get quite different results from testing the same sample at the same time.

PT scheme organizers should ensure that they are aware of any possible effects on the stability of the sample during the period of the PT round, and should ensure that the standard deviation for proficiency assessment takes any possible instability into account. Participants should also take part in PT frequently enough to build up sufficient data to monitor their own results over time.

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