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REVIEW

PROFICIENCY TESTING IN THE FOOD MICROBIOLOGY LABORATORY

DIANE ROBERTS

PHLS Food Hygiene Laboratory, Central Public Health Laboratory, London, United Kingdom

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Laboratories offering a quality service for the microbiological examination of food must implement a quality assurance system. An effective system should, in addition to daily quality control of procedures, consumables, and equipment, include external laboratory accreditation to a recognised standard, the use of validated test methods, and participation in an external quality assessment (proficiency testing) scheme.

External quality assessment (EQA) is a system in which samples of known but undisclosed content are introduced into a laboratory's routine testing procedure, in other words, a challenge to those procedures. There are a number of EQA schemes available to food microbiology laboratories, either offering freeze dried mixtures of organisms as simulated foods or preweighed portions of spiked dried foods.

There are a number of important requirements for an effective EQA scheme and a range of benefits from participation to a laboratory. These are discussed in the light of experience with the development of a specific scheme.

Key words:
external quality assessment scheme, laboratory accreditation, quality assurance system

For laboratories to produce accurate and reliable results from the samples they examine it is essential that a Quality Assurance system is in place. Quality assurance, as defined by International Organization for Standardization (ISO) (1), is all those planned and systematic actions necessary to provide confidence that a product or service will satisfy given requirements for quality. Such a system comprises two major parts: Quality Control which covers the tangible procedures performed in the laboratory on a regular basis to ensure that all aspects of the daily work are under control

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and Quality Assessment. Quality control will include monitoring of procedures, consumables, equipment, temperatures of incubators, refrigerators, and staff performance. In order that a laboratory can assess its performance the second part must be implemented: participation in a Quality Assessment or Proficiency Testing scheme whereby the Quality Control procedures are challenged by the examination of samples of known but undisclosed content. The EU Additional Measures Food Control Directive (2) stipulates that official laboratories, that is, those undertaking food examination for legislative purposes, should be accredited to the EN 45000 series of standards (3), should use validated methods, and should participate in a proficiency testing scheme. In addition to challenging procedures within a specific laboratory, proficiency testing is also a means of assessing performance against that of other laboratories.

The operational techniques and activities that are used to fulfil requirements for quality are also referred to as Analytical Quality Control (AQC) (4) and can be differentiated into three lines of checking as outlined in Table 1.

Table 1 *Analytical control in microbiology*

Line of checking	Responsibility	Frequency	Purpose
First	Analyst	High	All aspects of analysts under control and consistent over time.
Second	Person independent of the analyst	Less frequent	Different analysts or equipment produce similar results. Individual results not biased.
Third	Laboratory management	Regular intervals	To ensure inter-laboratory standardisation.

The first line of checking is a means of self control by the analyst, but it should be supervised by the direct superior responsible for setting criteria and defining action plans. It should be included with every series of analysis. First line checks can be divided into those to be undertaken a) before the analysis (samples, equipment, media, filters, and reagents), b) during the analysis (noting of all information that becomes available such as temperature, anaerobic conditions, confirmation rates, colonial appearance, background flora, etc.) and, c) in addition to the analysis. The latter would include internal quality control procedures such as examination of additional samples with known characteristics, parallel plating, procedural blanks, positive and negative control samples, colony counts on different volumes/dilutions, use of control charts, and use of sufficient colonies for confirmatory tests.

The second line checks are implemented to assure reproducibility between different analysts or equipment, during training of new workers, and evaluation of established staff in order to maintain standards of subjective interpretation. Such second line checks would include, a) duplicate counting (randomly selected samples) by the same person to provide the counting error under repeatability conditions and by different persons, thus including both random and systematic components to the

variation. These will allow relative differences or standard deviations to be computed, b) duplicate analytical procedures to test the whole quantitative procedure by using duplicate samples and then plotting control charts, and c) intensified quality control tests as listed for first line checking.

The third line checks should be supervised by the quality assurance officer and include participation in an EQA scheme (proficiency testing) and the use of certified reference materials (CRMs) (5, 6). In the former, the samples are examined by different laboratories, the results interpreted retrospectively by the central organisation, and the performance compared with other participants. It is a flexible approach whereby participants apply their own laboratory methods. With CRMs, all laboratories follow a strict protocol and the certified value is valid only for the applied method. Results obtained with other methods can be compared with the certified values.

EXTERNAL QUALITY ASSESSMENT (EQA) OR PROFICIENCY TESTING (PT)

The focus of this paper is EQA/PT. The requirement for such schemes and the types of scheme available will be discussed with specific emphasis on those provided by the Public Health Laboratory Service (PHLS), UK, in the area of food microbiology.

EQA can be defined as an independent assessment of the competence of laboratories to perform tests accurately and precisely, providing a challenge to the effectiveness of the quality system of a laboratory and adherence by staff to that system. The PHLS organises microbiology EQA schemes for food, water, and clinical laboratories. The latter scheme is well established and has been operating for more than 25 years (7). The food and water schemes were launched in the early 1990s. The PHLS food scheme (8, 9) was in part developed as a response to the requirements of food legislation and the need for mutual recognition of results between member states of the European Single Market. For the concept of mutual recognition of results there is a need for mutual recognition of microbiological standards, guidelines and specifications, the use of standard methods of known accuracy and precision, laboratories competent to perform the agreed tests accurately and precisely, and periodical, independent assessment of accuracy and precision of microbiological results (proficiency testing). It is the latter requirement to which EQA schemes are targeted. There are many benefits to be derived from participation in an EQA scheme beyond the requirement for official laboratories stipulated in the EU legislation (2). Such benefits are listed below:

- With Quality Control provides a total Quality Assurance package;
- Assessment of level of performance against an external standard of performance;
- Comparison of current and past performance;
- Comparison of performance with other laboratories;
- Identification of unsatisfactory performance (laboratory personnel);
- Method of demonstrating staff competence;
- Assessment of improvement over time;

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- Identification of declining performance and introduction of remedial action;
- Educational tool for training staff and improving performance;
- Performance of less familiar tests can be improved;
- New methods can be introduced;
- Source of help and advice;
- Strict confidentiality allows discussion of problems;
- Provides confidence to participants;
- May assist in the marketing of laboratory services.

In order to provide these benefits to laboratories there are also requirements of the providers of EQA schemes as listed below:

- Regular distributions;
- Stable, homogenous samples;
- Samples that mimic the condition and content of real food;
- Examination by the routine procedures in use in each laboratory;
- Requests for tests for the whole range of food examinations.;
- Assessment of results;
- Reports to participants giving both individual and overall results;
- Provision of repeat samples and technical advice;
- Complete customer confidentiality.

Although EU legislation (2) does not stipulate detailed requirements for EQA schemes, in the UK, the Department of Health, the Ministry of Agriculture, Fisheries and Food, and the UK Accreditation Service (UKAS) have produced an agreement (10) that sets out minimum requirements of quality assessment for food microbiological examination laboratories to comply with the additional measures Directive. These requirements include approval of EQA Schemes by the competent authorities, method by method accreditation, detection and enumeration tests where appropriate, a minimum of ten accreditation parameters (aerobic colony count, enumeration of Enterobacteriaceae or coliforms, *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* and detection of *Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp, and *E. coli* 0157), a minimum of 12 samples examined per year with determination of each of the parameters at least once per year and samples that reflect 'reality'. Thus the level, physiological condition and distribution of target organisms and background flora should be as close to that found in real food as possible.

EQA SCHEMES AVAILABLE

Table 2 summarises some of the schemes available to laboratories in the UK and elsewhere and the type of sample provided. Both the PHLS (8, 9) and the Nordic scheme (11) provide freeze dried mixtures of organisms at predetermined intervals and require participants to undertake a defined range of tests. The samples mimic real foods in their bacterial content but do not contain any food ingredients. The samples, as a result of their method of preparation, will contain sublethally injured organisms as would be found in the real food situation.

Table 2 *Some food microbiology External Quality Assessment (EQA) schemes available in Europe*

Name	Type of sample	Distribution (sample) per annum	
PHLS Food EQA Schemes[a]	Freeze dried mixture of target organism and background flora	Food (standard & extended) Dairy Shellfish Non-pathogen	6 (min 12) 4 (min 8) 2 (min 4) 3 (min 6)
Smart QA[b]	Real foods, sterilised and inoculated in multiple discrete foci with stable populations of organisms	As required by participant	
Quality in Microbiology (QM)[c]	Bacteria spray dried, diluted in sterile food/skimmed milk powder and used to prepare final sample	As required by participant	
Food Examination Performance Assessment Scheme (FEPAS)[d]	Realistic food-based test materials containing target organisms and background flora	As required by participant	
Nordic Proficiency Testing Scheme[e]	Freeze dried mixtures of organisms		3 (12-15)

a) Public Health Laboratory Service, Food Hygiene Laboratory, London, UK
b) Laboratory M, Bury, Lancaster, UK
c) Quality Management, Bury, Lancaster, UK
d) Ministry of Agriculture, Fisheries and Food, Central Science Laboratory, Norwich, UK
e) National Food Administration, Uppsala, Sweden

The other three schemes (Smart QA, QM, and FEPAS) (12, 13) prepare mixtures of organisms in a food-based test material, often in a dried, granular form. The samples are in a pre-weighed quantity and all the material provided must be examined in contrast to the PHLS and the Nordic Schemes where the samples are reconstituted to a set volume and portions removed for the various tests. Although the schemes providing food-based samples have annual programmes of distribution of various samples, the customers may select the frequency of distribution and the microbial content of sample according to their specific needs in relation to the range of products examined and the tests undertaken. The PHLS and the Nordic Schemes offer a total annual package covering the full range of food associated organisms.

THE PHLS FOOD EQA SCHEMES

The PHLS currently offers five schemes to its customers. Two of these, the Extended and Standard Schemes, cover a wide range of food associated organisms. The Shellfish and Dairy Schemes are specifically tailored to the test requirements of EU legislation for molluscan shellfish (14) and milk and milk-based products (15), respectively. Laboratories undertaking limited Quality Control testing or those located on food

production sites require samples that do not contain pathogens. The non-pathogen scheme has been produced in response to that requirement. Table 2 gives the frequency of distribution and numbers of samples.

The Standard Scheme

This scheme, offering six distributions of samples (2 samples every 2 months), covers the minimum 10 parameters required by the UK Departments and UKAS agreement mentioned earlier (10) and is thus appropriate for »Official Laboratories« (as defined by EU legislation) (2) and those offering a basic range of food microbiology tests. Samples are despatched to participants with a direct request to examine for specific parameters. A fixed period – usually three weeks – is allowed for completion of the tests using the laboratory's own methods and for the return of the results to the organisers. Once the closing date is past, an »intended results« letter is sent to all participants followed by a report and score sheet prepared on completion of the analysis of results from all laboratories. The »intended results« letter gives the participants the opportunity to request a free repeat sample for further examination should they have failed to isolate the target organism or achieve the correct enumeration value at the first attempt. The reports issued enable participants not only to monitor their own performance but also to compare it with that of other participating laboratories.

Before and during each distribution samples are also examined in the Food Hygiene Laboratory Quality Assessment Section on a weekly basis. The aim is to provide the organisers with a) confidence that the samples are of the required standard and b) are not contaminated with unwanted organisms. The tests also furnish data (reference results) with which participants' results can be compared. A wide range of laboratory methods and media are used in an attempt to encompass those that may be employed by participants.

Extended Scheme

The Extended Scheme is similar to the standard scheme in relation to distribution and reporting but it differs in that the examinations covered are broader and an investigative approach is needed. In addition to the minimum ten parameters, other, less frequently encountered pathogens such as *Vibrio parahaemolyticus* and *Yersinia* spp are included and so is the enumeration of a wider range of pathogens and indicator organisms. Instead of a direct request to perform specific tests, a background history of the sample is given and the participant must decide which organism(s) to seek. This scheme is thus suited to Public Health Laboratories and those offering a broader range of examinations. Table 3 describes a typical extended scheme sample.

Shellfish Scheme

This scheme is aimed at laboratories that examine raw bivalve molluscs and other shellfish for classification of harvesting beds and for end-product testing as required by EU legislation (14). The examinations covered are enumeration of *E. coli* (harvesting bed and end-product testing) and detection of *Salmonella* spp (end-product testing).

Table 3 *A typical extended scheme sample*

Sample history:	A refrigerated ready-to-eat pasta dish to which roast chicken was added. The chicken may have been undercooked. The pH of the product was 6.9; the temperature on sampling was 4.7 °C	
Request:	Examine for significant pathogens Assess the aerobic colony count Assess the *E. coli* count Return the results by the date stated on the forms	
*Sample content:		cfu/g
	Campylobacter jejuni	10^4
	Salmonella liverpool	10^3
	Pseudomonas aeruginosa	10^3
	Escherichia coli	10^3

* This information is mailed to participants on the dates the results are due

Dairy Scheme

Initially, this scheme was formulated to address the requirements of the EU Milk and Milk-based products Directive (15) which covers the plate count (30 °C), pre-incubated plate count, enumeration of coliforms and *E. coli* and *S. aureus*, and detection of *L. monocytogenes* and *Salmonella* spp. However, it was quickly recognised that many dairy laboratories are located on production sites and would not receive samples containing pathogens. Two options were therefore offered: the full range of tests and a non-pathogen option that covered plate counts, enumeration of coliforms and *E. coli*, and, additionally, spoilage organisms such as lactic acid bacteria and yeasts and moulds. This range of tests is typically undertaken by an on-site laboratory testing for product quality control purposes. In order for the samples to simulate more closely a dairy product, a portion of milk powder is despatched with the sample for rehydration and use in reconstituting the EQA sample (16). Duplicate samples are included when tests require preincubation at a specific temperature.

SCORING OF RESULTS

In order to assess the performance of participants a score can be allocated to each participant's results. This may be based on a straightforward correct, partially correct, incorrect, or misleading result on a presence/absence test for a specific pathogen or on how close an enumeration is to the expected result. Not all schemes offer a scoring system, although all include a statistical treatment of results. The PHLS has scored results since the inception of the first scheme as it provides a simple and useful indication of performance over time and can alert organisers and laboratories to occasional or continuing poor performance.

A good scoring system must enable a distinction to be made between good and poor performing laboratories over time. It must be weighted according to the impor-

tance of various aspects of the identification of microorganisms and be easy to apply, analyse, present and interpret. It must also be portable across samples to enable scores to be combined and comparisons to be made within and between laboratories over time.

The current PHLS Food EQA scoring scheme is based on distribution of a small number of points to the results. For detection and identification this is: intended result 2, partially correct (correct genus, sample not referred for complete identification) 1, incorrectly negative or result not returned 0, unexpected pathogen –1 and sample not examined no score. For enumerations the points are allocated according to whether they fall within the middle 80% of counts, 2, in the lowest or highest 6–10% of counts, 1, unless the enumeration falls within ± 0.5 $\log_{10}$ median count when it is upgraded to 2, or in the lowest or highest 5% of counts, 0, or whether results are not returned, 0. Thus after each distribution a participant will receive a score sheet giving the sample number, intended result, participants report, and score for the samples in that distribution and also a cumulative score for a defined set of samples, the mean score from all reports returned by laboratories testing those samples, and the number of standard errors the participant is above or below that mean. Participants can readily assess their performance and compare it with those of the other participants after each distribution. There are limitations with this simple two point system in that it is difficult to score when the tests required include enumeration of a specific pathogen or when more than one target organism is included. Scoring on presence/absence is sometimes insufficient as for certain pathogens microbiological standards specify maximum levels for safety. Thus there is a need to indicate how close the count was to the intended result. A scoring scheme is therefore being developed which will use a larger number of points to be allocated to difference aspects of the results and which will give a better record of performance.

ASSESSMENT OF PROFICIENCY

Proficiency can be measured in a number of ways. Some schemes calculate Z scores for enumeration data as defined in the international protocol (17). Z scores are derived from the reported results, the assigned (true) result calculated using the robust or consensus mean of all data and the target value for standard deviation derived from collaborative trial data. The Z scores for all participants can then be plotted on a graph and performance limits added (e.g. Z score=± 2 Satisfactory, $\pm > 2$ to 3 Questionable, $\pm > 3$ Unsatisfactory). The PHLS schemes present enumeration data in the form of simple bar charts of all participants' results with those from samples examined in the QA section superimposed for comparison. The 5, 10, 90, and 95 percentiles and corresponding scores are marked on the chart for easy interpretation.

As yet, there is no internationally agreed method of statistical analysis appropriate for the presence/absence data. Most schemes assign an assessment on the basis of correct, false positive, or negative results.

When undertaking performance analysis in microbiological quality assessment there are a number of factors which must be taken into consideration. Each sample

is a dynamic biological system in which the presence of different groups of organisms can affect the growth and the behaviour of others. To mimic the real situation, some pathogens such as *Salmonella* and *Campylobacter* will be present at low levels only and ease of isolation will be affected by their physiological condition and the presence of competing flora. The stability of the EQA samples must also be taken into account and the variations in the methods employed by different laboratories. The PHLS has introduced a system of management of poor performance aimed at satisfying the requirements of EU legislation and those of UKAS. The system relates only to the ten parameters required by the competent authorities (10) and thus includes data from both qualitative and quantitative examinations. It does not make a judgement on results from a single distribution but on a continuous series of results; that judgement is made by microbiologists. The system includes all EQA customers and not just »official laboratories«, although UK official laboratories must participate in all distributions. Confidentiality is maintained throughout the process. A rolling assessment is made for the last 3 and 6 distributions and performance is based on achievement of 70% of the maximum score. If the performance is below 70% after three distributions, participants receive a letter advising them of their low score and including general advice. If the performance remains below 70% after six distributions, a formal letter is sent with a list of general points and an offer of assistance in overcoming problems. Moreover, EQA participants are reminded that poor results could affect accreditation status as well as their status as an official laboratory. Accredited laboratories will be able to provide performance data for inspection by their accreditation body and evidence of action taken when performance falls below an acceptable level.

OTHER ASPECTS OF THE PHLS SCHEMES

The various distributions of samples, particularly those where multiple samples of the same target organisms have been included, have been used to obtain useful data from customers relating to their satisfaction with the service provided or to the methods employed. Such information has allowed the schemes to be modified and refined and for QA testing to be focused on the methods most appropriate to participant usage. The methods questionnaires also reveal the wide range of small modifications introduced by different participants whereby they are no longer following the »standard« method.

Worldwide distribution

The PHLS Food EQA Schemes provide samples to over 500 participants in 26 countries. The freeze dried format means that the samples are stable and can withstand the vagaries of the various postal and/or courier distribution systems. Most laboratories participate on an individual basis with a simple route of communication from and back to the PHLS. However, collaborative systems have been set up where a single laboratory in a country acts as the focal point for receipt of samples and reports from the PHLS for forwarding on to other laboratories in that country. That laboratory also

undertakes the translation of documentation and collation of results for transmission back to the PHLS which then produces the final report for translation and distribution by the collaborator. The advantage of this collaborative approach is that it can considerably reduce the costs of participation to individual laboratories as a proportion of the work normally performed by the PHLS QA section is taken on by the collaborating centre. Thus individual schemes can be tailored for collaborators according to the requirements of participating laboratories and the level of resources available.

REFERENCES

1. *International Organization for Standardization (ISO)*. ISO 8402 Quality management and quality assurance – Vocabulary. Geneva: ISO, 1994.
2. *Council of the European Communities*. Council Directive No 93/99/EEC on the subject of additional measures concerning the official control of foodstuffs. Off J Euro Commun 1993; L 290,24.11.93:14–17.
3. *European Committee for Standardisation (CEN), European Committee for Electrotechnical Standardisation (CENELEC)*. European Standard EN 45001 (BS7501): General criteria for the operation of testing laboratories. Brussels: CEN/CENELEC, 1989.
4. *Lightfoot NF, Maier EA, eds*. Microbiological Analysis of Food and Water, Guidelines for Quality Assurance. Amsterdam: Elsevier, 1998.
5. *Mooijman KA, in't Veld PH, Hoekstra JA, et al*. Development of microbiological reference materials. Commission of the European Communities. Report EUR 14375 EN. Brussels: Community Bureau of Reference, 1992.
6. *in't Veld PH*. The use of reference materials in quality assurance programmes in food microbiology laboratories. Int J Fd Micro 1998;45:35–41.
7. *Snell JJS*. The UK National External Quality Assessment Scheme for Microbiology. In: Roberts C, Kelsey MC, eds. Microbiology Accreditation and Quality Assessment Schemes in the UK, Measuring up to the Standards. London: Association of Medical Microbiologists, 1992:18–20.
8. *van Netten P, Roberts D, Russell J, et al*. The PHLS Food Microbiology Quality Assessment Scheme. In: Roberts C, Kelsey MC, eds. Microbiology Accreditation and Quality Assessment Schemes in the UK, Measuring up to the Standards. London: Association of Medical Microbiologists, 1992:28–30.
9. *Roberts D, van Netten P, Russell J, et al*. The PHLS Food Microbiology Quality Assessment Scheme. Third World Congress Food Borne Infections and Intoxications. Proceedings, Vol 1. Berlin: Institute of Veterinary Medicine, 1992: 526–31.
10. *Ministry of Agriculture, Fisheries and Food, Department of Health, United Kingdom Accreditation Service*. Agreement on the implementation of the quality standard for food control laboratories as required by EC Directives 89/397/EEC and 93/99/EEC. London: MAFF Joint Food Safety and Standards Group, 1998.
11. *Peterz M*. Laboratory performance in a food microbiology proficiency testing scheme. J Appl Bact 1992;73:210–6.
12. *Scotter SL*. Proficiency testing in food microbiology. MAFF Central Science Laboratory 'Quality Assessment Scheme'. Fd Sci Technol Today 1996;10:227–30.
13. *Scotter SL*. Proficiency testing for food examination. Int Food Saf News 1997;6:10–1.
14. *Council of the European Communities*. Council Directive No 91/492/EEC laying down the health conditions for the production and the placing on the market of live bivalve molluscs. Off J Euro Commun 1991;L 268,24.9.91:1–14.
15. *Council of the European Communities*. Council Directive No 92/46/EEC laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk based products. Off J Euro Commun 1992;L 268,14.9.92:1–32.

16. *Gough NL, Russell JE, Roberts D.* The PHLS Dairy Products External Quality Assessment Scheme. PHLS Microb Dig 1997;14:131–4.
17. *International Organization for Standardization (ISO), Association of Official Analytical Chemists (AOAC INTERNATIONAL), International Union of Pure and Applied Chemistry (IUPAC)*. International harmonised protocol for proficiency testing of (chemical) analytical laboratories. Pure Appl Chem 1993;65:2123–44 (also published in J AOAC Int 1993;76:926–40).

Sažetak

PROSUDBA VRSNOSTI LABORATORIJA ZA ISPITIVANJE MIKROBIOLOŠKE KAKVOĆE HRANE

Laboratoriji koji daju valjane usluge za ispitivanje mikrobiološke kakvoće hrane moraju provoditi program osiguranja kakvoće. Uz svakodnevnu provjeru metoda, kakvoće potrošnog materijala i opreme, učinkoviti program osiguranja kakvoće također uključuje akreditaciju laboratorija prema određenom standardu, uporabu validiranih metoda i sudjelovanje u vanjskoj prosudbi kakvoće laboratorija (prosudba vrsnosti). Vanjska prosudba kakvoće laboratorija sastoji se u uključivanju uzoraka poznatog ali neobjavljenog sadržaja u rutinska laboratorijska ispitivanja radi potvrde valjanosti upotrijebljenih postupaka. Postoji više programa za vanjsku prosudbu kakvoće mikrobioloških laboratorija za ispitivanje hrane u kojima se primjenjuju smrznute mješavine mikroorganizama slične onima u hrani ili uzorci osušene hrane prethodno nacijepljene poznatom količinom mikroorganizama.
U radu se navodi nekoliko važnih zahtjeva za učinkovitu vanjsku prosudbu kakvoće te niz pogodnosti za laboratorij koji sudjeluje u toj prosudbi. O tome se raspravlja na osnovi iskustva stečenog pri razvoju specifičnih programa.

Ključne riječi:
akreditacija laboratorija, program za vanjsku prosudbu kakvoće, sustav osiguranja kakvoće

Requests for reprints:

Diane Roberts, Ph.D.
PHLS Food Hygiene Laboratory,
Central Public Health Laboratory,
61 Colindale Avenue, London NW9 5HT, UK
E-mail: *DRoberts@phls.nhs.uk*