

UK NEQAS for Microbiology, P O Box63003, NW9 1GH

Tel: +44 (0)20 8905 9890 Fax: +44 (0)20 8205 1488 email: [organiser@ukneqasmicro.org.uk](mailto:organiser@ukneqasmicro.org.uk) web: <http://www.ukneqasmicro.org.uk>

***Investigation of failures with  
EQA specimens***

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## Introduction

These notes are intended to provide participants in the UK NEQAS for Microbiology with some guidance on the investigation of failures with EQA specimens. The examples given are mostly from bacteriology, but the basic principles will apply to other areas. The author appreciates that many participants in the schemes have considerable recent experience of clinical microbiology and their laboratories have excellent quality systems. However, the appropriate response to problems revealed by EQA schemes may be outside of the experience of more recent participants, and these notes may be helpful.

External quality assessment is only one component of a quality system. Some definitions may help to define the relationships between the components.

- *Quality assurance* is the total process whereby the quality of laboratory reports can be guaranteed.
- *Internal quality control (IQC)* comprises the processes carried out to check that media, reagents and equipment are performing within specifications.
- *External quality assessment (EQA)* is the challenge of the effectiveness of a laboratory's quality system with specimens of known but undisclosed content.

A comprehensive quality assurance system will cover such areas as provision and control of standard operating procedures, education and training, planned maintenance and calibration of equipment, monitoring of response times. Many laboratories are formally accredited to acknowledge conformance with defined and objective quality standards such as those in ISO 17025 or ISO 15189.

Results of consistently good quality can be expected only when all the components of a quality system are in place. This seems a daunting task to those starting along the quality path, but the process is incremental, and every quality component added will help to improve the situation. However, the following limitations are self-evident:

- EQA is not a substitute for other components of the quality system, and in particular, EQA cannot replace IQC.
- EQA is of limited value without at least some of the other quality components such as adequate documentation, training of staff and IQC.
- Most failures with EQA specimens are a result of inadequacies in the other components of the quality system.
- EQA tells you that you may have a problem, it does not solve the problem.

## Management issues

EQA is a tool to help senior laboratory staff to identify possible problems in the laboratory. The aim is to provide management with an insight into the quality of the routine work of the laboratory. The following qualifying factors apply:

- EQA results only give an insight into routine results if EQA specimens are treated in the same way as routine specimens.
- If EQA specimens are given special treatment, EQA results may be good but nothing will be learnt about the quality of the routine service and patient care will be compromised.

There are several ways in which EQA specimens may be given 'special' treatment. They may be handled by more senior grades of staff or subjected to a greater range of diagnostic procedures than normal or results may receive special scrutiny before. These practices should be discouraged by laboratory management.

Management need to be sensitive in the way that they deal with failures with EQA specimens. Problems may arise from failures in the quality system rather than from errors by staff. If too heavy handed an approach is taken, staff will become defensive and will take more effort with EQA samples in future to avoid further criticism. Quality systems will not be effective unless the laboratory staff feel a sense of 'ownership'. For this reason, it is essential to involve staff closely in the process of quality system development. A positive approach to EQA, with regular meetings to discuss results and emphasis on the educational aspects will do much to reassure staff.

## How reliable are the UK NEQAS specimens

An initial response to failure with an EQA specimen may be 'there was probably something wrong with the specimen'. It is of course not possible to guarantee that every single sample of a batch of EQA specimens is representative of the batch as the only way to do this would be to examine every specimen before issue. However, stringent manufacturing practices, past experience with the stability of the specimens and sampling of the batch within UK NEQAS does provide good assurance that it is unlikely that a participant will receive an unrepresentative specimen. Even if a participant does receive such a specimen by chance, it is statistically extremely unlikely that they will receive a series of them.

With serum specimens, occasional artefactual problems are encountered with some kits due to changes in the matrix caused by rethrombinisation of plasma or dilution. Such problems are usually resolved by joint investigation by the manufacturer and UK NEQAS for Microbiology.

## **The philosophy of UK NEQAS for Microbiology**

The schemes are educational; they are not designed to be punitive or for use in licensing of laboratories. The specimens are mostly straightforward; they are not designed to be 'tricky' or to catch people out. The specimens will mostly reflect what you are likely to find in routine specimens in your laboratory; however the proportion of 'positives' is of course greater in the EQA specimens. Occasionally, a more difficult or unusual specimen may be included in order to give participants the opportunity to gain experience.

The objective of the schemes is to allow participants the opportunity to learn from failures. It is not expected that participants should obtain correct results with all specimens; in fact a 100% success record might be viewed with some suspicion!

## **On receipt of the specimens**

- Decide if the specimen is relevant to your normal practice. It is possible that some types of specimen or specimens with a particular clinical history would not be examined in your laboratory. In such cases the reply form should be entered/marked as 'not examined'. You may decide to examine the specimen for training or educational purposes but there is little point in reporting the result as it will not mirror your routine procedures.
- Ensure staff read the information sheet and web instructions carefully for special procedures and any changes from previous distributions.
- Try and make sure that the specimens are handled by the normal staff using routine protocols. Treat them in the same way as you would an equivalent patient specimen with a similar clinical history.
- Keep records of your results to help investigation of any errors.
- Keep any remaining specimen material in case that you need to investigate failures, this may save you time as an alternative to requesting a repeat specimen from UK NEQAS. Serum and fungal specimens can be stored at 4°C. If a -70°C freezer is available, many of the bacteriology, and molecular will remain stable at this temperature if transferred soon after reconstitution. Stability of the more delicate pathogens cannot be guaranteed and a repeat specimen from UK NEQAS may be needed.

## **On notification of the intended results**

Intended results are published on the secure area of the website usually the day following the close of each distribution. E-mail notification of the posting of the intended results is available to participants supplying their e-mail address. Compare your copy of the reply form with the intended results and decide if any repeat specimens need to be ordered to investigate discrepancies. Decide if immediate action is necessary or whether investigations should wait until your individual distribution report arrives (usually, within 7 to 10 days). Generally, it is best to perform at least an initial investigation as soon as possible because as time passes it becomes more difficult for the staff involved to remember events.

## On receipt of your report

### Reviewing failures with individual specimens

- Read the distribution report to see if many other participants failed with the specimen and for relevant comments made in the report.
- Review all your results with the laboratory staff including successes as well as failures. Ensure that results are available to all, preferably through a 'quality notice board'.
- Keep records of your reviews and the reasons for any decisions made. File them where they can be retrieved for future reference. The availability of such records will demonstrate your responsible approach to quality to accreditation bodies.
- If there are discrepancies, decide if they are relevant.
  - In some cases you would not expect ever to encounter the organism included in your laboratory.
  - In other cases, you would not normally look for the organism in that type of specimen because you consider it not relevant. You may wish to review your procedures in such cases to satisfy yourself that they are still in line with common practice.
  - Full typing, e.g. of viruses, may not be relevant to your normal practices.

If you are satisfied that the results are simply not relevant to your circumstances, then you do not need to take any further action other than recording the reasons for your response.

- The score awarded is intended to provide you with a management tool. It allows comparison of your results with that of the 'average laboratory' and also serves to bring individual discrepancies to your attention. The allocation of a score is a means of bringing to your attention differences between your report and what has been designated as the 'correct' result. In most cases there is little argument about the appropriateness of the score but there will always be differences in laboratory practice both within and between countries that will mean that the score may not be totally applicable to a particular situation. The scoring scheme is regularly reviewed by an advisory panel of UK microbiologists to ensure that it remains relevant to UK practice. Scheme organisers are always pleased to hear the views of participants on problems with the scoring system but in many cases it has to be recognised that no scoring scheme can be universally applicable or relevant.
- If you did not order a repeat specimen when you viewed the intended results, decide if one might be useful and if so order it from UK NEQAS.
- Define what the actual error was. Did you fail to isolate a pathogen, fail to identify, misidentify or, misquantify it, report an incorrect sensitivity profile, fail to detect an antibody, report a false positive result or an unexpected result? Was the probable cause of the error one of technique or of interpretation? Depending on the answer to these questions, you may need to look at your procedures for specimen processing and preparation, your culture conditions, your tests

(characterisation, IF, EIA, PCR...), your criteria for interpreting technical results, or reporting procedures.

- Try to follow an audit trail. Can you identify what batch of media, antisera or any test kit you were using? Was it within the specified shelf life? What were the IQC results on that batch of media or kit? In the case of serological and molecular assays, were the controls of the batch in which the EQA specimens were tested within specifications? Who handled the specimen and who authorised the report? Any laboratory with a serious interest in the quality of patient care needs to ensure their quality systems can provide these answers both for EQA specimens and, more importantly, with patients' samples.
- If you have saved the original specimen or have obtained a repeat specimen then re-examine it. Are there still problems? Is the pathogen growing poorly on your media? Are agglutination or any test results/reactions weak or doubtful? Are your controls behaving as expected? Have you used negative and positive controls? Have you a collection of similar pathogens or similar clinical samples that you can try out? UK NEQAS may be able to provide you with some similar material or organisms if requested.
- False positive results may be due to contamination caused by carry over between adjacent specimens. For any procedure the results for negative controls should be reviewed. For molecular assays, consider results of both extraction step and test run negative controls. With serological assays, check that the condition of the specimen appears satisfactory, e.g. is it cloudy perhaps indicating precipitation of proteins or microbial growth. A common cause of false positive results is incomplete washing of micro-wells and washers should be checked and maintenance procedures reviewed.
- False negative results may be caused by failure to add the specimen to the test system. Some pathogens are very labile and you should review whether the specimen was tested immediately upon reception and/or reconstitution. With serological assays, the presence of micro-clots in the specimen may lead to false negative results, especially in automated systems. Such events should be rare with UK NEQAS samples. For molecular assays consider the extraction of the sample; examine the results obtained with the extraction positive control and of the internal control.
- For quantitative molecular assays how did your results compare with other users of the same assay? What extraction method was used? What volume of sample was extracted? Was your result close to the limit of detection/quantification of your assay?
- Procedures and schedules for maintenance, calibration and monitoring of equipment such as incubators, pipettes, washers, readers, microscopes, immuno-assay systems, extraction and amplification platforms should be reviewed.
- Failures with slides for immunofluorescence are often caused by incorrect set-up and maintenance and inadequate control of the fluorescence microscope.

- Transposition errors are common with EQA specimens (and also presumably, with clinical specimens). Check that either specimens or results have not been transposed during testing or reporting.

Failures with EQA specimens are almost certainly a reflection of similar failures with routine specimens and you will wish to act to prevent such failures compromising patient care.

Appropriate action may include:

- Introducing or refining IQC procedures
- Training or retraining of staff
- Introducing or refining stock control
- Altering or formalising specimen work up procedures
- Revising standard operating procedures (or methods manuals)

It must always be borne in mind that single EQA specimens may not be representative of the material that is routinely examined in a laboratory. Strains of bacteria for example can vary considerably in their growth requirements, in their antigenic structure and in their biochemical characteristics. For this reason it is unwise to make major changes such as in the supplier or formulation of tests or media on the basis of results with single EQA samples. Such changes may give better results with the particular EQA specimen but worse results with the majority of clinical specimens. Before such changes are made it is necessary to confirm that the problem revealed is general in nature and this will require further investigation with clinical samples.

### Reviewing cumulative performance

As well as providing details on performance with current specimens, the individual laboratory reports also give details of cumulative performance over a period of time, normally 6 or 12 months. The data provided shows your cumulative score with the specimens examined in this period, the mean score derived from the results of all participants, and the number of standard errors that your cumulative score is above or below the mean. This provides a useful assessment of your laboratory's performance relative to that of other participants. By the very nature of a mean, it is inevitable that some participants cumulative scores will be below it on occasions. Consistent performance below the mean, or downward trends are probable evidence of continuing quality problems that need to be addressed. Examine the plots of your performance rating over time to identify the trends. A cumulative score of more than 1.96 standard errors below the mean probably indicates significant quality problems that need to be resolved in order to safeguard patient care.

### **On receipt of annual reports and record sheets of performance**

Every year, the results obtained between the periods April to March are reviewed. Participants also receive record sheets showing their results in all specimens examined in this period. This is a good opportunity to review performance over the year and to see if there are any problem areas with particular pathogens or samples that have been overlooked. Again, all staff should have access to these results.