Arh hig rada toksikol 1998;49:371–378

REVIEW

QUALITY ASSURANCE IN AQUATIC BIOLOGY – A USER'S PERSPECTIVE

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Received 3 November 1998

The essential elements of developing biological quality assurance systems are presented in terms of the 4M principles. These relate to methods, manpower, materials, and machines. The use of international or European standards is recommended where such standards exist. Users must be sure that these are appropriate to their specific needs as standardisation can require considerable compromise. Examples of limitations in international standards are given with reference to the coliform isolation by membrane filtration, *Daphnia magna* acute toxicity test and the luminescent bacteria test. The criteria for the selection and use of national methodologies are considered using macroinvertebrate, macrophytes, imposex, and the oyster embryo bioassay as examples. In recognising that the main resource in science is the skill, training, and dedication of the scientists themselves, the United Kingdom has developed a quality initiative aimed at best utilising the human resource, the so-called Investors in People (IIP) initiative. This contains the essential elements of any quality system: commitment, planning, action, and evaluation. Quality aspects of the materials and the machines used in biological analyses are briefly considered.

Key words:
audit, Investors in People, machines, manpower, materials, methods, standardisation

Quality Assurance is an essential element of producing reliable, relevant, and reproducible biological data on which sound management decisions can be based, thereby maximising the impact of financial resources invested, as well as ensuring consistency between laboratories and indeed countries.

Presented at the AOAC INTERNATIONAL Central Europe Subsection 5th International Symposium on Interpretation of Chemical, Microbiological and Biological Results and the Role of Proficiency Testing in Accreditation of Laboratories, Varaždin, Croatia, 21–23 October 1998.

In contrast with chemical and physical methods, biological quality assurance techniques have until recent times been largely ignored. Increasingly within the European Union (EU) there is a demand for adequate quality assurance of biological analyses primarily driven by the requirements of legislation. For example, the EU Directives relating to urban waste water treatment, nitrates, habitats, and bathing water as well as the proposed water framework all contain provisions for biological measurement for compliance assessment.

Without comparability of methods and their equitable application there is limited potential for the objective application of the mandatory criteria defined in EU legislation. Ultimately, without fully addressing these issues the effective management of water quality issues becomes an impossible task.

This has been recognised within the EU and biological standards under elaboration within CEN/TC 230 Working Group 2 – Biological Methods will include guidance on the key quality assurance criteria specifically relevant to individual methodologies. In addition, a new Task Group under an Austrian chairmanship has recently been established to elaborate general quality standards applicable to generic biological analyses.

The development and maintenance of robust quality systems is, therefore, essential, despite the acknowledged cost of their establishment and maintenance. The cost of operating a quality system has been estimated as being as high as 20% of staff time, however, in the experience of the author this can be streamlined to something around 10% of staff time. This still represents a considerable investment. The alternative is the production of biological data of dubious quality with the risk of wasting considerably more in terms of resources in the implementation of scientifically indefensible water management programmes.

General considerations in elaborating biological quality systems

Inevitably, the demand for quality assurance in biological analysis is customer led usually in response to legislative or regulatory requirements. The starting point inevitably involves bench marking comparisons with what are regarded to be the prestige reference organisations expert in specific fields. This then forms the target in achieving excellence in specific areas of biology.

The emphasis then changes to the development of a quality system and quality management system. Where possible, accreditation should be sought by external agencies. The organisation is only as good as external audit shows it to be.

In the UK, the premier external quality assessment organisation is the United Kingdom Accreditation Service (UKAS). UKAS not only defines in detail the requirements of quality systems but also audits against these standards, using independent auditors to ensure compliance. These audits consider all of the essential elements of effective quality systems. The key elements are internal audits and reviews, equipment, methods, sampling, sample handling, staff competency, training, measurement traceability, the working environment, recording, subcontracting, and customer complaints. This degree of audit rigour ensures the scientific credentials of theOrganisation and the Quality System operated.

At an operational level, this seemingly complex matrix required for effective biological quality assurance can be distilled into four key elements; the 4M principles of Manpower, Methods, Materials, and Machines.

THE 4M PRINCIPLES

Methods

Ideally, biological methods should be widely used, well validated, and reproducible between laboratories and countries. The precision should be well defined and appropriate to the measurement required as well as being suitable for regulatory purposes. In the EU context, the use of standards elaborated by ISO or CEN is usually a prerequisite of the legislation. However, when working with international standards, users are advised to ensure that they are appropriate for the purpose intended, as well as being up to date, as occasionally compromises have been made in order to ensure that standardisation can be accomplished.

For example, the isolation of coliform bacteria by membrane filtration (1) contains the classic elements of compromise due largely to the divergence of methodologies between North America and Europe. The existing standard ISO 9308 defines a coliform organism as being capable of forming colonies at either 35±0.5 °C or 37±0.5 °C on a selective and differential lactose culture medium with the production of acid and aldehyde within 24 hours. The implication is that a coliform can grow anywhere between 34.5 °C and 37.5 °C which says little for standardisation in microbiology.

In the same standard temperature (1) tolerances for thermotolerant coliforms are given as 44±0.25 °C or 44.5±0.25 °C. Clearly the 0.25 °C variation cannot be justified when the actual limits quoted in the standard lie between 43.75 °C and 44.75 °C.

This issue is being addressed by a revision of this standard and the new limits for coliforms are quoted as 36±2.0 °C and thermotolerant coliforms as 44±0.5 °C. The temperature limits are at least scientifically defensible, if still somewhat generous.

A quite separate problem arose with the 1996 revision of the acute toxicity testing with *Daphnia magna* standard ISO 6341 (2). As part of the revision, the tolerance limits of the reference toxicant, potassium dichromate were reworked using routinely collected validity data submitted from practising laboratories. This was subjected to the same statistical rigour (3) as would be appropriate with ring-test data. The new limits were set at an EC_{50} of 0.6–1.7 mg/L.

As a result of an exceptionally large data set produced by one laboratory, these limits were artificially biased and due to the fact that an inappropriate statistical summary had been used, a clone that had been used for many years for regulatory purposes suddenly did not meet the validity criteria. In turn this compromised the regulatory role of at least some of the practitioners despite the fact that the clone had performed satisfactorily in the past.

Communication between the standards subcommittee responsible for the standard with ISO/TC 147/SC 7 – Precision and Accuracy, identified the appropriate statistical methodology. The data were reworked and new reference toxicant limits of EC_{50} were defined as being 0.6–2.1 mg/L. The apparently problematical clone now complied with the standard and a technical corrigendum has been issued in order to correct the previous error.

The luminescent bacterial inhibition test, ISO 11348 (4), also presented a number of standardisation problems in that there were three possible test systems based individually on freshly culture, liquid dried, and freeze dried bacterial cultures. Different

countries have favoured different methodology options largely due to the availability of commercial preparations.

It is acknowledged that the results of the three potential test systems are not directly comparable and that this in turn required better evaluation of precision data. Additionally, the incorporation of three separate methods into a single standard is in breech of ISO rules.

Corrective measures have now been undertaken. The standard is now in three separate parts. No single commercial package is favoured ensuring the neutral role of ISO. The validity criteria have been updated and the standard is likely to be published in late 1998.

Where international standards are not yet available, national methodologies should reflect the state of the art and be highly standardised and subject to the same quality audit rigour as international standards. Examples here include imposex, oyster embryo bioassay, macrophyte surveying, and river classification systems based on benthic macroinvertebrates.

Even here the analyses should be subject to the normal elements of quality assurance, specifically including aspects of rigorous internal quality control, where appropriate, and always including participation in external proficiency schemes. In all instances, the data degenerated must be traceable in order to ensure that any non-conformances can not only be detected, but can be traced back to source thereby enabling continuous improvement in performance.

In the United Kingdom considerable effort has been expended classifying rivers based on their invertebrate fauna as a means of assessing water quality and detecting temporal changes, as well as quantifying the effects of water quality improvement programmes. While international standards have been under development for some considerable time, they are unlikely to be finalised in the next few years.

To address this shortcoming, detailed national methodologies have been elaborated for sampling and the subsequent analysis. Quality assurance methodologies have been developed involving internal AQC and external audit supported by both internal and external auditing of all part of the analysis.

The United Kingdom has sought to develop macrophyte surveying and classification methodologies in response to a demand from an impending EU Water Framework Directive. While attempts are being made to standardise surveying methodologies, the acceptance of standardised classification methods are at this moment in time the subject of considerable debate and the need for targeted research is readily acknowledged by the European standardisation body, CEN/TC 230. Hence it is essential, in the interim period, that the United Kingdom adopts scientifically defensible methodologies based on sound quality assurance principles.

The result is that the UK not only demands the regular training of its river macrophyte surveyors, but supports the evaluation of the macrophyte surveyors through an IdQ examination provided by the national expert body, the British Natural History Museum.

In turn, quality assurance is assessed by the re-surveying of river reaches by national experts and the potential to compare specimens collected with voucher specimens. The involvement of national experts in creating an ethos of quality in river macrophyte surveying is critical to the success of the work and ultimately the aim will be to achieve accreditation that will withstand the rigours of external audit to UKAS standards.

Tributyltin, which has been traditionally used as an anti-fouling paint for boats and fish farming cages, has been described as the most toxic substance ever deliberately introduced to the aquatic environment (5). Its action as an endocrine disruptor has readily detectable effects on the females of some species most notably through imposex in the dogwhelk, *Nucella lapillus* (6, 7), shell thickening in the Pacific Oyster, *Crassostrea gigas* (Thunberg) and high mortalities in oyster larvae (8).

Imposex in *Nucella* results in the development of male characteristics in the female gastropods. The females grow a penis with a subsequent loss of fecundity. The degree of imposex is determined by comparing the volumetric size of the female penis with that of the males from the same communities, expressed as a percentage which is the Relative Penis Size Index (RPSI) calculated from the equation:

$$\text{RPSI} = \frac{\text{Mean female penis length}^3}{\text{Mean male penis length}^3} \times 100$$

The methodology has gained UK national acceptance through inclusion in the National Marine AQC scheme and recently European acceptance through being the first biological test to be included in Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME). Indeed the imposex test is the first biological test to be included in the QUASIMEME proficiency scheme. UK interlaboratory trials of the methods have assisted with this broader acceptance, supported by a national survey of all marine waters by the national reference laboratory.

In the United Kingdom the oyster embryo bioassay has been widely accepted as a sensitive method of detecting trace toxicants in marine waters where concomitant chemical testing would be prohibitively expensive. The exposure of the test organism is during a period of intense cellular activity and division where the effects of pollutants readily interfere with normal larval developmental processes.

The bioassay is particularly sensitive in that even in the absence of toxicants the failure rate in larval development is high. The need for effective quality assurance in this context is even more apparent, particularly as this test in an integral component of the UK National Marine Monitoring Programme. Laboratories inputting data must achieve demonstrable proficiency in order to have data included in the national data base.

This is achieved by the use of a highly standardised United Kingdom methodology produced from a protocol developed by International Council for Exploration of the Seas (ICES). The survival rate of the juvenile oysters must meet defined criteria and the response to reference toxicants, zinc and phenol, must be within the respective ranges of 0.1–0.5 and 100–320 mg/L to meet the validity data for the test. The bioassay has been ring-tested for ICES and the UK National Marine Monitoring Plan – laboratories must have no flagged results for inclusion of data into the national database.

Manpower

The most important resource in science is the scientists themselves. Good quality outputs rely on the commitment, knowledge, training, professional pride, and ultimately the job satisfaction of the individual scientists. Additionally, the inherent variability in biological measurement when compared with conventional chemical analysis

is widely acknowledged and hence the need for structured and focused, high quality training, retraining, assessment, and review is critical. The enhanced contribution of the individual to the business objectives is an obvious asset to the corporate organisational aspirations.

This has given rise to the »Investors in People« initiative in the United Kingdom. The essential elements of this initiative are not dissimilar to those of any quality assurance system: these include the commitment, the planning, the actions required, and, finally, the evaluation of the corporate input in terms of resource.

As with any move towards improved quality there needs to be the commitment. The vision of the organisation must be promoted in the context of staff training and development thereby stimulating the involvement of the staff as well as the support of management. The benefits to the individual and the organisation need to be stressed within the context of the corporate aims. These activities are aimed at maximising the contribution of the individual and generate improved communications throughout the organisation.

As with any quality improvement programme there needs to be careful strategic planning which is written into the overall quality ethos where training and development are carefully identified and regularly reviewed. Equally, resources must be quantified in setting the objectives of both the organisation and the individual. Future actions in training and development are based on regular reviews of the outputs from structured training and personal development.

The actions required in meeting staff development must be based on the common philosophy of the involvement of the individual in the success of the organisation. In turn, individual motivation is reliant on encouragement through perceived opportunity, although this opportunity is, in turn, constrained by the resources available and the priorities established by organisational needs.

The subsequent evaluation of the contribution of the improved knowledge, skills base, and attitude needs to be evaluated in the context of addressing key organisational issues. Costs-benefit evaluation targets the effectiveness of future training.

As with any quality scheme, the performance in audit against defined criteria is essential. Documentary evidence of not only the aims and actions but also the results of the evaluations must be robust enough to satisfy internal and external inspection, as must the expectations and perceptions of the staff involved. The concept of quality must include an aspiration for continuous improvement which in turn requires continual assessment.

Materials

Central to the accuracy of any biological test are the materials used. Where quality standards are not met and met consistently there is the clear potential for wasting a scientist's time due to the need for additional controls and quality checks. This is becoming less and less acceptable especially when it is the responsibility of the manufacturers and suppliers to ensure the suitability of materials for use.

The quality of not just the materials but their traceability from source to point of use must be established within a quality organisation, which in turn places demands on manufacturers and suppliers to demonstrate their commitment through accreditation, preferably to ISO 9001 standards.

Machines

The machines used in biological testing must be adequately maintained and regularly calibrated. Systems need to be in place to ensure that malfunctions are rapidly detected in order that remedial actions can be undertaken promptly, placing a minimum demand on re-working testing.

Traceable calibration of parameters such as temperature, pressure, and weight to international standards can only enhance the quality of biological measurement especially when supported by a range of internal and inter-laboratory accuracy checks. Reference to the luminescent bacterial test and standard microbiological instrumentation serves as exemplar.

The detail of documentation is, of course, expensive and time-consuming, but it will ensure the robustness of the quality systems against internal and external audit. Additionally, where deficiencies are encountered, the effects on tests can be quantified through traceability and hence, effective remedial actions are achievable.

CONCLUSIONS

Quality assurance is a continuous improvement process of detecting problems, finding solutions, and putting in place effective remedial actions. This is achieved through the regular auditing of the quality system, both internally and externally, with concomitant involvement in not only external proficiency schemes, but also with the development of internal quality assurance systems.

REFERENCES

1. *International Organization for Standardization (ISO)*. ISO 9308-1: Water quality – Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* Part 1: Membrane filtration method. Geneva: ISO, 1990.
2. *International Organization for Standardization (ISO)*. ISO 6341: Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (*Cladocera, Crustacea*) – Acute toxicity test. Geneva: ISO, 1996.
3. *International Organization for Standardization (ISO)*. ISO 5725-2: Accuracy (trueness and precision) of measurement methods and results – Part 2: Basis method for the determination of repeatability and reproducibility of a standard measurement method. Geneva: ISO, 1994.
4. *International Organization for Standardization (ISO)*. ISO 11348: Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 1: Method using freshly prepared bacteria; Part 2: Methods using liquid-dried bacteria; Part 3: Method using freeze-dried bacteria. Geneva: ISO, 1998.
5. *Goldberg ED*. TBT: An environmental dilemma. Environment 1986;28:14–44.
6. *Gibbs PE*. The use of the dogwhelk *Nucella lapillus*, as an indicator of tributyltin (TBT). J Mar Biol Ass UK 1987;67:507–23.

7. *Smith PJ, MacVeigh M.* Widespread organotin pollution in New Zealand coastal waters as indicated by imposex in dogwhelks. Mar Poll Bull 1991;22:409–13.
8. *Claisse D, Alzieu C.* Copper contamination as a result of anti-fouling paint regulation. Mar Poll Bull 1993;26:395–7.

Sažetak

UTVRĐIVANJE KVALITETE U BIOLOGIJI VODENIH SUSTAVA – SA STAJALIŠTA KORISNIKA

Temeljne postavke u razvoju sustava osiguranja kvalitete bioloških analiza prikazane su u principu 4M: metode, ljudski potencijal (»manpower«), materijali i mehanizacija. Primjena međunarodnih ili europskih standarda preporuča se u svakoj prilici gdje ovi postoje. Korisnici moraju biti sigurni da su ovi standardi primjereni njihovim specifičnim potrebama s obzirom na to da bi međunardni standardi mogli zahtijevati značajne prilagodbe. Na primjerima izolacije koliformnih bakterija membranskom filtracijom, testom akutne toksičnosti *Daphnia magna* i testom toksičnosti s luminiscentnim bakterijama pokazana su ograničenja u međunarodnim standardima. Kriteriji za odabir i primjenu nacionalnih metodologija pokazani su na primjeru makroinvertebrata, makrofita, imposeksa i bioloških pokusa na embriju kamenice. Uzimajući u obzir da je glavni problem u znanosti iskustvo, uvježbanost i privrženost poslu znanstvenika, Ujedinjeno Kraljevstvo razvilo je inicijativu za razvoj kvalitete sa svrhom najprikladnijeg iskorištenja ljudskog potencijala, tzv. »*investicija u ljude*«.
Ovaj pristup sadržava temeljne elemente svakog sustava kvalitete: privrženost, planiranje, djelovanje i vrednovanje. Aspekt kvalitete materijala i opreme upotrebljavane u biološkim analizama samo je kratko razmatran.

Ključne riječi:
investicija u ljude, ljudski potencijal, materijali, metode, oprema, osiguranje kakvoće, standardizacija

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