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Review

External Quality Assessment in Microbiology. Pitfalls and Tips

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Abstract

The External Quality Assessment (EQA) is mandatory for the accreditation of medical laboratories. In Romania, this is a requirement of the National Insurance House based on the 1301/2007 Order of the Ministry of Health. Besides, the EQA providers must be accredited according to the specific standard ISO 17043:2010. Some issues can be considered as pitfalls when referring to the full and correct implementation of EQA requirements. It is important to acknowledge them and to know how to overcome them. Some of these pitfalls are presented and the ways in which they can be solved are discussed, as follows: the selection and the monitoring of the EQA provider; the real needs of the laboratory in terms of frequency, type of scheme, number of samples; the way in which the performance of the total testing process is assessed and the means of presentation of the results of the assessment. For microbiology EQA, additional important aspects are isolation or identification of the microorganisms with or without clinical significance, inhomogeneity and short stability of the samples, testing of appropriate antimicrobials, detection of the antimicrobial resistance mechanisms, detection of critical or borderline levels of antibodies, antigens or nucleic acids. Laboratory types (for inpatients or outpatients, for infectious diseases, general hospitals, for public health surveillance or for reference activities) should be taken into account when comparing the performance of each laboratory with overall performance. The EQA is a useful tool for continuous quality improvement, knowledge and experience playing a major role.

Keywords: External quality assessment, EQA provider, microbiology laboratories, microorganisms, microbiology proficiency testing, pitfalls.

Резюме

Външната оценка на качеството (ВОК) е задължителна за акредитирането на медицинските лаборатории. В Румъния това е изискване на Националния застрахователен институт въз основа на Заповед 1301/2007 на Министерството на здравеопазването. Освен това, провеждащите ВОК трябва да са акредитирани по специфичния стандарт ISO 17043:2010. По отношение на пълното и правилно прилагане на ВОК се установяват някои недостатъци. Важно е те да се съобщят и да се знае как да се преодоляват. В статията са представени някои от тези недостатъци и са обсъдени начините за решаването им, както следва: изборът и мониторингът на провеждащия ВОК; реалните потребности на лабораторията по отношение на честота, тип на схемата, брой проби; начинът, по който се осъществява цялостният процес на проверката и представянето на резултатите от оценката. За ВОК в микробиологията допълнителни важни аспекти са изолирането и идентификацията на микроорганизмите, които имат или нямат клинично значение, липсата на хомогенност и кратката стабилност на пробите, тестването на подходящи антимикробни средства, определянето на механизмите на резистентност, определянето на критичните или граничните нива на антителата, антигените или нуклеиновите киселини. Когато се сравняват резултатите на всяка една лаборатория с общите нива, трябва да се имат предвид и типовете лаборатории (за болнични и извънболнични

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пациенти, за инфекциозни заболявания, за обединени болници, за надзор на общественото здраве или за референтни цели). ВОК е полезно средство за непрекъснато подобряване на качеството, като знанието и опитът имат водеща роля.

Introduction

The continuous raising of the number and types of analytes and laboratory tests performed in medical laboratories, including microbiological laboratories, has become nowadays a challenge for these.

How can we ensure the confidence in the results and in the quality of these tests, regardless of where they are performed or of how many resources (equipment, personnel, money) the laboratory has? One highly valuable tool is the regular participation in the External Quality Assessment (EQA). EQA is mandatory for the accreditation of medical laboratories according to the specific standard ISO 15189:2012, being an essential part of the quality assurance of testing results. In Romania, this is a requirement of the National Insurance House based on the 1301/2007 Order of the Ministry of Health. Besides, the EQA providers must be accredited according to the specific standard ISO 17043:2010.

External Quality Assessment schemes, often named Proficiency Testing (PT), are probably the most important type of interlaboratory comparisons in the world of medical laboratories (Eurachem's Proficiency Testing Working Group, 2005). For sure, other types of interlaboratory comparisons such as the validation of methods and the determination of a reference value of materials are of equal importance and value in laboratory activity.

Providing an independent feedback on the quality of the total testing process, the results of the EQA represent not only a means for monitoring laboratory performance, but also the starting point for its improvement. This is very important for patients, for healthcare professionals, for accreditation bodies, as well as for authorities and for society in general. In this regard, the following purposes of EQA schemes can be mentioned: to assess and compare the reliability of laboratory performance on a national scale, to provide assurance to both physicians and the general public that laboratory diagnosis is of good quality, to identify common errors, to encourage the use of uniform procedures and standard reagents, to stimulate the implementation of internal quality control programs, even to take administrative measures (which may include revocation of the operating license) against substandard laboratories (De Vandepitte *et al.*, 2003). EQA also represents a valuable educational element, indicating either the success of staff training, or if additional training is required.

Like other specific activities performed in laboratories, EQA exercises are planned processes with objectives, resources, time framing, activities, results, indicators of performance, opportunities for improvement, in other words, they could be viewed under the Deming Cycle PDCA (Plan, Do, Check, Act). Formal development of a Proficiency Testing Plan provides a long-term "roadmap" for laboratories to ensure the continuous improvement of the quality of their services (Gust, 2003). For each step in this cycle there are some issues which represent not only challenges but even real pitfalls for both laboratories and PT providers, when referring to the full and correct implementation of the specific, already mentioned standards requirements. It is important to acknowledge them and to know how to overcome them. A real help in solving such aspects, both for PT providers and for laboratories, comes from the Eurachem guideline "Selection, Use and Interpretation of Proficiency Testing (PT) Schemes" (Mann, 2011) and the ILAC document "ILAC-P9:06/2014 - ILAC Policy for Participation in Proficiency Testing Activities" issued a few years ago.

Some of these pitfalls are presented below, together with short explanations and possible solutions or tips to avoid them.

Some problems concern both laboratories and PT providers while others are specific for only one category. There are also common issues for all types of laboratories and others are particularly relevant for microbiology laboratories.

Common Pitfalls and Tips

The first really difficult to solve issue is how the laboratories identify their real needs for EQA schemes: length and frequency, type of scheme, number of samples, and also if the matrices, the analyses, and their levels fit the routine measurements in the laboratory; all these must be carefully checked before signing the contract with an EQA provider, which must be able to accomplish them (Eurachem's Proficiency Testing Working Group, 2015). The key information should be found in the EQA leaflet or should be requested from the provider to help laboratories to choose those EQA

schemes that are best suited to their needs.

In terms of the length of the proficiency testing cycle and the frequency of participation, both legal provisions and guidelines in the field should be considered as minimum requirements (e.g. EA-4/18:2010, Guidance on the level and frequency of proficiency testing participation). In order to decide on a suitable level and frequency of EQA participation, a risk assessment should be performed (Eurachem's Proficiency Testing Working Group, 2016). If major or critical non-conformities on testing processes are identified (during an internal or external audit by the accreditation body), or the laboratory has recorded related complaints, or the results of previous EQA rounds are repeatedly unacceptable, the frequency should be increased.

The type of scheme (qualitative, semi-quantitative or quantitative) should be chosen in accordance with the test methods currently used in the laboratory.

The number of samples submitted for testing per exercise, the matrices, the number of analytes and the concentration levels which must be determined for each sample or analyte also depend on the methods used in the laboratory and should be similar to those of samples tested in the everyday practice of the laboratory (the performance of the methods, as it has been established in the validation/verification studies). The competence of the staff plays an important role in testing complex samples or samples with borderline levels of analytes. For training purposes, the samples with a reasonable number of analytes and clearly normal and pathologic levels must be chosen first, afterwards, the number of samples, the number of analytes/sample and the number of levels of analytes might be increased.

Another important common pitfall is the selection of an inappropriate EQA provider in terms of quality of test materials, methods used for assessing the performance of laboratories, turnaround time, means of presentation of the assessment reports and other relevant information, costs, etc.

The test materials are manufactured samples, having varied origins (human - desirable but often not available -, animal or artificial), more or less characterized, with target values determined, assigned (Reference Materials - RM) or even certified (as are the Certified Reference Materials - CRM). The samples can be produced by the EQA providers or by IVD manufacturers, both according to the recently issued ISO 17034:2016 standard. Their homogeneity and stability must be determined and guaranteed by the manufacturer (by means of validation studies) and must be checked by the EQA provider before each delivery of the samples to the participants in the EQA schemes (ISO 17043:2010). If the large dispersion of the results cannot be explained by other causes, the inhomogeneity or/and instability of the samples must be taken into account. If such events are repeated, it is more rapid and less costly for the PT provider to change the supplier or manufacturer of the samples. A less known aspect is the accompanying documentation of the samples: the quality certificates and the Material Safety Data Sheets. These include information on traceability, properties, the uncertainty of measurement, expiry data, and safety measures to be taken in case of incidents or accidents. A special concern for the EQA provider who is also the manufacturer of the samples is maintaining an accurate and complete documentation for each lot of samples, from primary raw materials to finished products (ISO 17034:2016).

The opportunity to report the results of testing by many ways (by mail, by email or online) should be appreciated by the laboratories as an advantage. The same opportunity must exist for submitting the assessment reports.

Processing of the results can be done with a dedicated software or manual, but the final judgment must be done by people with appropriate and experienced training, according to ISO 17043:2010 and ISO 13528:2015. A panel of specialists is desirable, as leaving the job to one single person might be subjected to mistakes.

How is the performance in the EQA scheme assessed and compared?

Besides the overall performance, it is desirable, mandatory even, in some circumstances, to perform the assessment of the performance by grouping the results on methods or equipment used in participant laboratories, by batches of reagents (this type of comparison is very useful for IVD manufacturers). Some international/national EQA schemes perform the assessment by grouping the results by countries/regions or by type of laboratories (clinical hospital laboratories, public health laboratories, etc.).

The ISO 15189:2012 standard used for accreditation of medical laboratories stresses that EQA schemes should "... have the effect of checking the entire process including pre- and post-examination procedures." The ability to assess the total testing process has a strong dependence on some elements of the design of the scheme (the amount, complex-

ity and clarity of the clinical information available before testing), on which the requested operations are to be performed by the laboratory in the pre-examination phase, and on how the results of the testing must be reported to the EQA provider. A significant amount of clinical information, not clearly stated, or a very summary information could be a real pitfall for the laboratory. A lack of information concerning some pre-examination operations (e.g. centrifugation of sample) or in the post-examination phase (e.g. the measuring unit for reporting the results) could have a negative impact on the results and on the performance of the participants.

The report forms for presenting the assessment results should not be neglected; both the general aspect and the content must be taken into account. Summary or detailed information, with or without explanatory notes (these having an important educational role), only tabulated data or color graphics in which the laboratory's results and the expected results are suggestively presented and easy to identify, the trend of the laboratory results (during the previous year) can make the difference between EQA providers.

Laboratory types (for inpatients or outpatients, for infectious diseases, general hospitals, for public health surveillance or for reference activities) and their dimensions (small or large, single, multi-site or network of laboratories) are also very important and these aspects should be taken into account when comparing the performance of each laboratory with overall performance.

Many providers organize regular meetings with the participants to discuss results and problems that have arisen. These meetings offer opportunities for education and training.

The shortest turnaround time is one of the factors that the laboratories rely on to quickly correct their deficiencies and improve their performance. An unexpected prolonged time for returning the assessment report could be a signal for a lower capacity or an overshoot. The expected results should be available immediately after the end of the reporting period (on the EQA provider's website) followed in the shortest time by the assessment results.

Although the financial aspect of EQA participation is an important asset in the selection of the EQA provider, the cheaper is not always the best choice and the most expensive is not necessarily a guarantee for the quality of services.

All the above-mentioned issues regarding the EQA provider should be considered for its monitoring by the laboratory. An acceptable score above the threshold (carefully established) should be mandatory to maintain the EQA provider on the list of agreed suppliers. At the same time, any doubt about the quality of services must be carefully investigated and any significant difference between the laboratory needs and the services provided should lead to the withdrawal of the respective EQA provider from the list.

More information about EQA schemes and providers can be obtained from the national accreditation body, from the EPTIS website or from other international organizations such as the WHO, Eurachem, Eurolab or EQALM. In Romania, according to the initial Order of the Ministry of Health no. 315/2005, all the EQA schemes and the providers for medical laboratories are notified to the Ministry of Health and can be seen on its website.

Pitfalls and Tips Specific for Microbiology EQA

In addition to common pitfalls, in the field of microbiology, there are other important issues that should be acknowledged. Specific problems can be identified, in sub-domains such as bacteriology, virology, immunology, parasitology, etc., but these are not going to be distinctly presented below.

The clinical relevance of the microorganism or microorganisms that have to be isolated and identified in the sample is one of the issues raised by Noble since 2002.

Having in mind the ISO standard requirement to use in EQA schemes samples similar to those tested in the routine practice, the provider should send to the microbiology laboratories many types of samples: with clinical or public health relevancy (classical pathogens, newly recognized or opportunistic pathogens, e.g. *Burkholderia cepacia*), without relevancy (pure culture or a mixture of nonpathogenic microorganisms - to test the ability to recognize negative specimens) or both (e.g. a mixture of *Shigella* and *E. coli*, to test the skill of a laboratory in isolating pathogenic microorganisms from a number of commensal organisms) (De Vandepitte *et al.,* 2003).

With regard to the number of samples per round, it is recommended to send at least 3 samples, of which at least one should contain at least 2 microorganisms, one pathogenic, and one non-pathogenic. This is recommended, of course, in the case of simulating samples from non-sterile sites (De Vandepitte *et al*., 2003).

Reference type strains (wild phenotypes, susceptible to many antibiotics, except intrinsic resistance), abnormal strains (e.g. lactose negative *E.*

coli) or strains recently isolated from clinical samples can all be used. For the latter, aspects such as ethics and confidentiality must be considered.

The density of microorganisms per sample is important to verify the sensitivity of the methods, samples with low levels of microorganisms being selected.

Samples can be supplemented with a variety of non-traditional challenges to extend testing to include a wider range of the pre- and post-analytic aspects of the laboratory cycle (Noble, 2002).

Other specific aspects are the inhomogeneity and relative short stability of fresh samples. Different types of prepared samples (stabilized, lyophilized, mixed with preservation ingredients) are currently used with an acceptable quality level in this regard. But commutability can no longer be questioned in this case.

Biosafety and biosecurity during EQA exercises should not be neglected, in order to prevent accidents and injuries of staffs belonging to the EQA provider, laboratories and companies involved in the transport of samples. Microorganisms of level 3 biorisk group (e.g. *Salmonella* serovar typhi) must not be used in EQA exercises (De Vandepitte *et al.*, 2003).

When manual methods of identifying microorganisms are used, it is impossible to verify whether the EQA samples have been introduced into the current working stream at the time of receipt, as other clinical samples, or whether the processing has been delayed for various reasons. It is also impossible to detect if the test has been repeated with the same method or others.

The tricky requirement to test the susceptibility to antimicrobials, using antibiotic panels recommended by EUCAST or CLSI guidelines, as well as inappropriate antibiotics (with intrinsic resistance, or not recommended for certain administration routes) meanwhile providing comments on the requirements (clinical case related), can assess not only the technical competence on examination phase, but also the knowledge on pre-examination and post-examination steps.

The detection of special antimicrobial resistance mechanisms can be requested (e.g. methicillin-resistant *S. aureus*) (De Vandepitte *et al.*, 2003), but this particular aspect must be announced in the prospect of the scheme before starting the exercise.

Gram Stain Competency Assessment

Although the Gram stain is one of the most frequent techniques used in clinical microbiology laboratories, it is still a cornerstone for beginners and sometimes as well for experienced staff. The lack of standardization and the poor control contribute to this. A recent study on 6,115 specimens examined in four clinical microbiology laboratories revealed discrepancies between laboratories (the Gram stain error rate varied between 0.4% and 2.7%, with Z-scores of 3.6 to - 3.1); the main factors contributing to errors were poor specimen quality, smear preparation, and interpretation of the smears. Discrepancies between stain and culture also occurred in approximately 5% of the cases, with only one-quarter of these discrepancies being the result of reading error (Samuel *et al.*, 2016). College of American Pathologists-accredited microbiology laboratories is required to have a policy that addresses "correlation of direct Gram stain results with final culture results" (Microbiology Checklist 7.28.15 requirement number MIC21530) (Thomson, 2016).

Another type of assessment of competency in Gram stain interpretation is a computer-based one, using multiple-choice questions. This assessment helps laboratories to identify the areas for continuing education in Gram stain interpretation (Goodyear, 2006). A large images collection for EQA scheme at European level can also be a very useful asset to improve the proficiency in Gram stain interpretation.

Special Concerns in pre-and post-Examination Phases in Microbiology

The technological evolution has allowed automation in the field of microbiology (e.g. microbial identification, antibiotic susceptibility testing, etc.) and processing, storage, and transmission of a huge amount of data, leading to the reduction of human errors, especially during the examination stage. Therefore, the attention of the EQA schemes providers is increasingly focused on the evaluation of the pre-examination and post-examination stages, both distinctly and within the framework of the total testing process.

In Romania, in the last few years, some EQA providers have developed microbiology schemes to assess the capability of labs to identify errors in the pre-examination and post-examination stages, based on questionnaires with multiple choice questions or with open answers regarding laboratory decisions/actions in different hypothetical clinical situations/cases. There is still a large dispersion of the scores obtained but with the tendency to reduce the differences and to improve the performances of

the participating laboratories year by year (unpublished data). This type of scheme is a useful tool for identification of gaps in the knowledge of the staff and also a form of training. The following advantages have also been identified: costs are reduced, the scheme is based only on the imagination and experience of scheme managers and experts without other expenses, the risk of contamination is eliminated, the schemes can assess the areas less evaluated through classical schemes, they enable an unlimited number of participants. But there are also disadvantages: the cases cannot be treated as real patient samples, and there is no control over consultation with other people or even with other participants. To some extent these can be diminished by Internet-based EQA schemes, using digital images of the samples and online questionnaires with an immediate feedback.

Conclusions

EQA, *per se*, is still an essential element of laboratory quality management and a useful tool for continuous quality improvement. Nevertheless, it requires and relies on the knowledge and experience of the laboratory staff.

In the field of microbiology, the EQA schemes have some peculiarities, of which the providers of such schemes must be aware of during the design of their schemes, and the laboratories must select the provider and those schemes which are best fitted to the current methods used in the laboratory. Providers and laboratories should both avoid the common and specific pitfalls mentioned above.

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