BAKI UNİVERSİTETİNİN XƏBƏRLƏRİ

№4 ***Təbiət elmləri seriyası*** ***2013***

UTC 581.19:577.162

QUALITY CONTROL (QC) IN RADIO IMMUNOASSAY FOR MEASURING THE HORMONES OR ONCOLOGY MARKERS IN CLINICAL LABORATORIES

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The main purpose of any Quality Control (QC) procedure, is the maintenance of quality patient care. To avoid misdiagnosis, the result of an immunoassay, must be accurate with sufficient confidence. The present discussion is limited to the role of QC in the objective analysis of errors which impair immunoassay results. In the case of tumor markers like prostate-specific antigen (PSA), the results of control sera should be compared with the target value of a known reference laboratory. The activity which managed and run by the user laboratory is known as internal quality control (IQC) but those which run by different laboratories, commercial, national and international, is called as external quality control (EQC). The purpose of external quality control is to achieve a consensus of results and thus facilitate the pooling of information between different centers and countries. So, the QC provides an assurance of the validity of a diagnostic test and a guarantee of accuracy.

Key words: tumor marker, IQC, EQC, immunoassay

Introduction:

Prior to the 1960s, measurement of biologically active molecules could only be achieved by physiochemical methods or bioassay. The technical complexity, frequent insensitivity and expense involved severely, limited progress in both clinical medicine and research.

At the time, the control of biological systems by hormone receptor interactions and transport of hormones in proteins, such as: Thyroxine binding globulin, were well established phenomena.

Both, share a common ligand receptor principle which provide the basis of a fundamental technique, the protein- or ligand- binding assay [1].

Such methods theoretically promised simple, specific and sensitive analyses with universal applicability, given two essentials:

- High affinity, specific receptors which bind the analyte.
- A means of detecting biomolecules in very low (nano mole – pico mole) concentrations.

The first requirement was met by the application of antibody as receptor which can, in practice, be raised to any molecule (antigen) of biological interest. The second essential was met by the use of radioactive label, a conceptual advance which resulted in immunoassays. Berson and Yallow in the USA published the first radio immunoassay (RIA) for insulin in 1960.

At first, immune assay were originally developed in the field of endocrinology which extended the scale of assay a further 10^3 –fold into the pico mole range [1, 5]. Whole new areas of study were opened up. The technique was simple and the samples could be assayed directly, with no pretreatment, and at a single dose. The universal applicability has been demonstrated by the way in which other disciplines have developed immunoassays for their own applications. It has now become indispensable in immunology, oncology, virology and microbiology, drug testing and many areas of medical research.

Validity, limitations and accuracy of assay

It is of paramount importance that the results of any determination are quoted with sufficient confidence. Part of this guarantee is an assurance of the validity of the assay. In any binding assay the interaction between receptor and ligand is due to a mutual recognition analogous to a lock and key. In the case of immunoassay, molecular structures called epitopes are recognized specifically by antibody. Immunoassays may therefore be thought of as structural assays and contrast with functional assays such as bioassay [2,6].

A few researchers would tend to agree that the bioassay is somehow better than immunoassay because of inherent functional specificity as the immunoassay may measure non-functional immune reactive material. The corollary is that an immunoassay should be validated by reference to a bioassay. The problem with this approach is that because the two systems are based on different principles and are validated by different criteria they are not strictly comparable. It is better to accept that structural assays may measure molecules without direct biological activity,

But that such measurements yield information which is of use.

There are, however, important limitations attached to immunoassays which should be born in mind when interpreting results. Major complications can arise because of the heterogeneous nature of both antibody and many analytes.

Analytes, especially proteins, carry a number of epitopes, some of which may be shared with related molecules. Cross reaction may occur producing a blurring of the assay and ambiguities.

Another drawback arises directly from the binding assay principle which depends upon the conformation (shape) of both the receptor and ligand. The conformation of proteins and other biomolecules is particularly susceptible to the physiochemical environment and to enzymes such as proteases, which may be present endogenously or produced by contaminating microorganisms [4]. It should also be understood that immunoassays are equilibrium not endpoint determinations. The Ab-Ag interaction is a reversible, non-covalent interaction,

and slightly changes in any of the assay constituents can produce pronounced effects on the results [3].

Ideally, all valid assays should give the same answer for a given sample,ie the true value, the actual amount of analyte present. An assay which gives the true value is said to be accurate [6].

Moreover the extent to which data deviate from the true value is also dependent on errors.The limitations outlined above make it no surprise that immunoassays are more prone to variability than most other techniques. Rondomly occurring errors, eg variable carry over on the outside of a pipette tip, affect the reproducibility or precision of results,while a systemic error, eg an incorrectly adjusted pipette, will produce a consistent displacement of data or bias from the true value. Accuracy is dependent on both types of error, statistically:

$$\text{Accuracy} = \sqrt{\frac{SD2+bias2}{n}}$$

Where SD is the standard deviation of the distribution of replicated determinations (precision) and n is the number of replicates. In practice we separate the precision and bias, instead of considering overall accuracy, and analyse each component separately [6].

Objective of quality control

The ultimate goal of any quality control (QC) procedure in the clinical laboratory is, first and foremost, a maintenance of quality patient care. The present discussion is limited to the role of QC in the objective analysis of errors which impair immunoassay results. When operating correctly QC will flag-up suspect data which can then be discarded or interpreted with caution. Once a failure has been detected the source(s) of errors can be pin-pointed and rectified.

QC also acts at the manufacturing level- the selection of antisera, the integrity and purity of labeled tracer or antibody and the correct standardization of calibrants are all factors which can, and do, contribute to errors in the finished assay. QC may be thought of as a diagnostic assurance.

The laboratory staff using kits or matched reagents is primarily concerned with the first and second function of QC. Problem may lie with the expertise of laboratory staff (eg: reagents handling) or with malfunctioning equipment. The deficiency may then be rectified, eg by training staff. If faults are detected in the reagents themselves the problem should be referred to the manufacturer.

QC in practice falls into two broad categories: Internal QC. Which deals with management of errors within a single laboratory and External QC, which is primarily concerned with inter laboratory performance [2, 6].

Immunoassay practice and origin of error

For an appreciation of the function of QC procedures it is necessary to recognize where and how errors arise. In essence a single immunoassay batch consists of the following stages:

- Standard calibrants, quality control pools and unknown samples are dispensed to tubes or microtiter wells, usually in duplicate;

- Specific antibody and labeled tracer or labeled antibody is added and the mixture incubated for an appropriate time;

- Antibody “bound” and “free” fractions are separated;

- The label is quantified, eg by radioactive counting or enzyme-catalyzed colorimetry;

- A standard or dose-response curve is constructed, either by eye or with computer curve fitting, and the unknowns estimated by reading off the curve.

Within- batch errors occur at each stage. Poor pipetting is a common problem. It is important that the correct (most precise) pipette is used at each stage and it is critical that the same pipette is used for all standards and unknowns so that the precision and bias is uniform. A variable incubation time may introduce with in batch bias or drift. Incomplete separation of “bound” and “free” results in misclassification errors – a variable amount of “free” label associated with the “bound” gives imprecision in the dose.

Instrumental errors are governed by the physics of the label detection method. In the case of radioactive counting instrumental error is equal to the square root of the total number of counts, thus a count of 10,000 has an error of 1% which is usually satisfactory as procedural errors are commonly >3%. Subjective construction of the standard curve may introduce bias, this problem can be overcome by the use of computer curve fitting methods. Finally, unquantifiable errors such as blunders will occasionally occur [4,6].

A second assay batch, with different unknowns, would follow the same format with similar but probably not identical errors at each stage. The results may be a different precision and overall bias, ie between0batch variability, which must be within acceptable limits (see below). The bias could be significantly affected by factors such as incorrect use of standards, deterioration og reagents, altered incubation, equipment failure and inappropriate curve fitting.

Meanwhile, a neighboring hospital might be processing similar samples by another assay with a quite different method bias!

Internal QC

a) within batch errors

Standard curve parameters are the front line of QC. Binding at zero analyte(B_0), non-specific binding (NSB), the dose equivalent to 50% binding (ED_{50}) etc. are characteristic of any assay system and provide a simple and immediate check for errors. Blunders or omissions can be identified without using sophisaticated error analysis; eg: in a limited reagent assay if too little antibody is added due to a dilution error both B_0 and ED_{50} would fall, but if the tracer quality had deteriorated B_0 would be less and ED_{50} would remain constant.

The errors associated with immunoassay are dose dependent. Thus the coefficient of variation (CV%, the SD expressed as a percentage of the mean in

the response varies with different concentration of analyte, so that the equivalent dose errors, are not constant across the analyte range. The formal analysis of this phenomenon is expressed as the precision profile (figure 4), introduced by Ekins [4]. The usefulness of within-batch precision for QC purposes is, however limited in practice by the problem of attaining sufficient data.

In routine assays it would be unusual to do more than triplicates, but statistically around 100 data or more per assay are really necessary. Precision profile themselves are imprecise unless sufficient data are given. Nevertheless profiles are of much more use than other precision parameter. Drift is generally time-ralated and related to systematic and sometimes progressive error. It is easily detected by the inclusion of replicate 'drift control tubes' such as QC pols, in the assay. An analysis of variance follows: if within replicate variance is constant but between replicate variance is consistently higher drift is indicated.

b) between-batch variability

The use of QC pools is the preferred method of monitoring between assay reproductivity. QC pools are prepared from HIV antibody and HBsAg negative samples or by spiking virologically screened serum with analyte, and are stored under conditions at which no deterioration occurs. Commonly QC pools are supplied lyophilized and, upon reconstitution, stored at -70°C. Usually three pools are used – at the two extremes and in the middle of the dose range.

The mean, SDs and CV% are calculated from about ten or more consecutive assay batches, each of which have satisfied with in –batch QC criteria. For each pool the means becomes target value and control limits, often ±2SD, are set. Routine QC charts are then constructed (figure 5).

Given the data for three QC pools, 'out of control' criteria can be set for consideration of batch rejection, such as the starred assay in figure 5. There is always a 'grey area' involved when making such decision. For example, if one pool is +2SD, one on target and one -2SD, poor within-batch precision may be making assessment of between-batch bias difficult. In constrast in all pools +1SD is a good indication of bias. In addition to flagging-up aberrant assays the chart also provides an index of long term consistency [1,3 and 6].

External QC

Between -laboratory variability occurs in any measurement where a systematic error is present. In the early days of hormone immunoassay huge biases were observed and, at the time, the supra-regional assay service (SAS) saw the need for a concensus of results. Clearly where bias is abolished the pooling of information between laboratories and centers is greatly facilitated and improved patient care is likely to follow. External bias assessment is based on establishing a target value by a universally accepted reference method against which other methods are compared. The United Kingdom external

quality assurance scheme (UKEQAS) evolved from the original activities of the SAS.

QC pools covering relevant dose ranges are prepared centrally and distributed monthly to participating laboratories; the samples are assayed and the results returned to the center. Data are then analyzed and the group mean, estimates of bias and variance and cumulative bias and variance for each method are reported. Laboratories are thus provided with an index of performance relative to their peers. It should be remembered that assessment of bias is obviously constrained by within-laboratory variability. External QC should act as an independent check on within-assay performance and advice on internal QC as and when problems arise.

Immunoassay techniques provide analyzes of unequalled sensitivity and specificity. Because of the biological nature of the reagents (antibody) and the characteristics of equilibrium assays, however, immunoassays are more prone to errors and, consequently, to imprecision and bias than are most other techniques in chemical pathology. The role of QC in identification and elimination of errors is therefore essential for maintain the optimum diagnostic performance of immunoassay [1, 6].

Conclusion:

RIA techniques provide analyses of unequalled sensitivity and specificity. Because of the biological nature of the reagents (antibodies or tumor markers like prostate specific antigen" PSA") and the characteristics of equilibrium assays, however, immunoassays are more prone to errors and, consequently, to imprecision and bias than are most other techniques in chemical pathology. The role of QC in identification and elimination of errors is therefore essential for maintaining the optimum diagnostic performance of immunoassay in clinical laboratories.

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KLİNİK LABORATORİYALARDA HORMONLARIN VƏ YA ONKOMARKERLƏRİN TƏYİNİ MƏQSƏDİLƏ RADİO İMMUNOLOJİ MÜAYİNƏNİN KEYFİYYƏTİNƏ NƏZARƏT (KN)

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XÜLASƏ

Klinik laboratoriyalarda müayinənin keyfiyyətinə nəzarət (KN) prosedurasının əsas məqsədi keyfiyyətli müalicəni təmin etməkdir. Diaqnozun düzgün qoyulması üçün immunoloji

müayinə dəqiq və kifayət qədər konfidensial olmalıdır. Təqdim edilən məqalə immunoloji müayinənin nəticələrinə ziyan vuran səhvlərin obyektiv analizində KN-in rolunun müzakirəsi ilə məhdudlaşır. Belə ki, onkomarkerlərin, məsələn, prostat-spesifik antigenlərin (PSA) təyini zamanı kontrol zərdabların nəticələri tanınmış laboratoriyaların müvafiq qiymətləri ilə müqayisə olunmalıdır. Laboratoriyanın öz fəaliyyəti keyfiyyətə daxili nəzarət (KDN), kommersiya, milli və beynəlxalq laboratoriyaların fəaliyyəti isə keyfiyyətə xarici nəzarət (KXN) adlanır. Keyfiyyətə xarici nəzarətin aparılmasında məqsəd nəticələrin konsensusuna nail olmaq və bununla da müxtəlif mərkəzlər və ölkələrin aralarında informasiya axınını asanlaşdırmaqdır. Beləliklə, KN diaqnostik testin dəqiqliyini təmin edəcək.

Açar sözlər: onkomarkerlər, keyfiyyətin daxili nəzarəti, keyfiyyətin xarici nəzarəti, immunoloji müayinə

КОНТРОЛЬ КАЧЕСТВА (КК) РАДИОИММУНОГО АНАЛИЗА В ЦЕЛЯХ ОПРЕДЕЛЕНИЯ ГОРМОНОВ ИЛИ ОНКОМАРКЕРОВ В КЛИНИЧЕСКИХ ЛАБОРАТОРИЯХ

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РЕЗЮМЕ

Основная цель процедуры контроля качества (КК) в клинических лабораториях заключается в обеспечении качественного лечения пациентов. Для того, чтобы правильно поставить диагноз, результаты иммуноанализа должны быть точными и конфиденциальными. Данная статья ограничивается обсуждением роли КК в объективном анализе ошибок, которые могут навредить результатам иммунологического анализа. В случае опухолевых маркеров, таких как простата-специфический антигена (ПСА), результаты контрольных сывороток следует сравнивать с соответствующими значениями известных лабораторий. Деятельность самой лаборатории называется внутренним контролем качества (ВКК), деятельность же различных коммерческих, национальных и международных лабораторий, выступает в качестве внешнего контроля качества (ВКК).

Цель внешнего контроля качества заключается в достижении консенсуса результатов и содействовании, тем самым, обеспечению информационного потока между различными центрами и странами. Таким образом, КК предоставляет гарантию обоснованности диагностического теста и гарантию точности.

Ключевые слова: опухолевые маркеры, внешний контроль качества, внутренний контроль качества, иммунный анализ

Redaksiyaya daxil oldu: 27.09.2013-cü il
Çapa imzalandı: 27.01.2014-cü il