

## Demands on reference materials for optimized quality control in tumor marker diagnosis

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*The accuracy and precision of tumor marker testing is of high clinical importance demanding the implementation of effective quality control procedures in the diagnostic laboratory. For the internal quality control the use of reference materials is obligatory. Several requirements should be met by the reference materials applied: In particular, they should be stable, easy to handle and of patient-like composition. Different sources of reference materials for tumor marker diagnostics have been applied in the past: individual patient sera, pooled patient sera, test kit controls, lyophilized external controls and liquid BIOREF controls. This article is focused on the different demands on tumor marker reference materials and how they are met by different sources of reference materials.*

### BACKGROUND

Reliable tumor marker diagnostics are of high importance especially in the follow-up of patients undergoing cancer therapies. For this purpose mainly immunological tests are applied which require an effective quality control using reference materials [1, 2]. The European Group on Tumor Markers (EGTM) recommended that several requirements should be met by the internal quality control in a tumor marker laboratory [3]: These recommendations include the use of reference materials with concentrations appropriate to the clinical application which should closely resemble authentic patient sera. The reference materials should be used in order to assess the reproducibility of the test system. Criteria like the Westgard-rules should be established for the acceptability of the internal quality control results [4]. The importance of using reference materials in tumor marker diagnostics has been shown by several proficiency studies in the past e.g. for the tumor markers CA 125, CA 19-9, SCC and PSA [5, 6, 7, 8, 9]. These studies revealed that the comparability of results obtained by different test systems can be very low. Additionally, the precision of test results in

some laboratories was rather bad. This observation was on the one hand due to mistakes of the laboratory staff on the other hand this was due to the test systems used in the laboratories. In conclusion, these studies showed that reference materials are of help for the comparison of test results obtained with different test systems and for the detection of imprecision especially due to lot to lot changes of the test kits used.

### REQUIREMENTS TO BE MET BY REFERENCE MATERIALS

In order to ensure an effective internal quality control in tumor marker diagnostics the reference materials used should fulfil a number of criteria which are summarized in Table 1.

One of the main issues demanded by the European Group on Tumor Markers (EGTM) as well as by the German Bundesärztekammer is that a control serum should closely resemble authentic patient sera [3, 10.] This implies that a control serum should consist of a human serum matrix and native human antigens. The use of controls consisting of buffered matrices and

chemically or biochemically treated antigens cannot be recommended since such artificial controls will not react like patient sera. For this reason the EGTM recommends the use of an authentic serum matrix control from a source independent from the test kit used.

According to the EGTM and the German Bundesärztekammer reference materials with antigen concentrations appropriate to the clinical application should be used [3, 10]. This ensures that the test system is working properly in the concentration range critical for diagnostics and therapy control of tumor patients. The EGTM recommends the use of negative, low positive and higher positive controls for tumor diagnostics [3]. The German Bundesärztekammer claims that at least two controls with different analyte concentrations have to be used [10].

Especially in the follow-up of tumor patients, which is a process taking years, it is of outstanding importance that the reference material used has a long shelf life. This

enables the laboratory staff to use one lot of the same control over years and to compare patient values obtained with the control values measured. The change of test kit lots as well as the change of the whole test system can be monitored using one and the same control. One prerequisite for a long shelf life is a high long-term stability of the reference material. The other prerequisite is that large batches of one reference material can be produced.

It is of big advantage when the reference material can be handled in an easy way. Liquid controls reduce mistakes and costs efficiently compared to lyophilized controls which have to be reconstituted and sometimes have to be stored in aliquots.

Stabilizers and other components of the reference material should not interfere with the test systems used. Especially artificial controls containing e.g. acetate, nickel, glycerine or bovine serum albumin can cause erroneous results as shown by O. Sonntag for some

tests on the Vitros test system of Ortho Clinical Diagnostics [11].

For security reasons the reference materials should be checked for infectious diseases. According to the European Guideline for in vitro Diagnostics (98/79/EG) products must be designed or checked in a way that the risk of infection for the laboratory staff is at least minimized [12]. It is advisable to check reference materials at least for HBsAg, anti-HCV, HCV-RNA and anti-HIV 1+2 since these are the established security checks performed on blood and blood products in transfusion medicine.

The German Bundesärztekammer claims that reference materials supplied with target values have to be used - at least for the analytes covered by the new directive for quality control of the Bundesärztekammer [10]. These target values have to be included into the evaluation of test performance of the internal quality control. The target values should provide a measure for the evaluation of the accuracy as well as for the precision of test results.

### COMPARISON OF DIFFERENT SOURCES OF REFERENCE MATERIALS

Several different sources of reference materials are applied for the internal quality control in tumor marker diagnostics like individual patient sera, pooled patient sera, lyophilized kit controls, lyophilized kit-independent controls and liquid BIOREF controls. The general characteristics of these different sources with respect to the

Table 1.  
Requirements to be met by tumor marker reference materials

human serum matrix
antigens of human origin
native antigens
high long-term stability
clinically relevant antigen concentrations
long shelf life
easy handling
stabilizer not interfering with test systems
certified absence of infectious diseases
target values

requirements which have to be met by reference materials in tumor marker diagnostics are summarized in Table 2.

Individual patient sera with known tumor marker concentrations are ideal controls with respect to the composition since they exactly correspond to the patient sera to be measured. The main disadvantage of these sera is that there is only a very small amount of such individual sera available. A long-term quality control cannot be performed with such sera. Additionally, these sera only contain the

analyte of interest in a clinically relevant concentration. Since the stability of most analytes is rather poor these sera have to be stored frozen in aliquots.

By pooling individual patient sera in order to obtain a pooled patient serum the problem of small batch sizes is minimized but not solved since the batch sizes are still low. In principle it is possible to produce multi-analyte controls by this method but the risk of unwanted protein interactions cannot be ruled out. Such a serum also requires storage in frozen aliquots

due to the poor stability of the analytes. The main advantage of the pooled patient serum is still that it is of patient-like composition.

Kit controls are unsuitable for long-term quality control, too, since they are only available as one batch for a short period of time. Additionally, the kit controls often consist of an artificial matrix since they are often derived from the kit calibrators. This means that they do not reflect the composition of a patient serum. In many cases the matrix is adapted to the test kit. Therefore kit controls can be

recommended to check for handling mistakes of the laboratory staff only.

The main advantage of the lyophilized kit-independent controls is that they are produced in big batches so that a long-term quality control in principle is possible. But in many cases batch reservations are still necessary in order to ensure to acquire one and the same lot over a longer period of time (years). Unfortunately, the stability of the analytes is rather low after reconstitution which makes it necessary to store the

Table 2 Characteristics of different sources of reference materials

Demands on ideal tumor marker control	Patient serum	Pooled patient sera	Lyophilized kit control	Lyophilized kit-independent control	Liquid BIOREF control
human serum matrix	yes	yes	often buffered matrix	often buffered matrix	yes
human origin	yes	yes	sometimes animal or recombinant origin	sometimes animal or recombinant origin	yes
native antigens	yes	yes	often (bio)chemical manipulations	often (bio)chemical manipulations	yes
broad analyte spectrum	no	no	no	yes	yes
clinically relevant analyte concentrations	difficult to realize	difficult to realize	yes	yes	yes
high (long-term) stability	no (only several weeks at +4°C)	no (only several weeks at +4°C)	no (only few months at +4°C)	yes (several years at +4°C)	yes (several years at +4°C)
long shelf life	no (only small lots)	no (only small lots)	no (only provided with specific test kit lot)	no (only provided with specific test kit lot)	yes
easy handling	no (requires aliquotation)	no (requires aliquotation)	no (requires reconstitution & aliquotation)	no (requires reconstitution & aliquotation)	yes
stabilizer not interfering with test systems	yes	yes	yes	yes	yes
certified absence of infectious diseases	no	no	yes	yes	yes
target values	no	no	yes	yes	yes

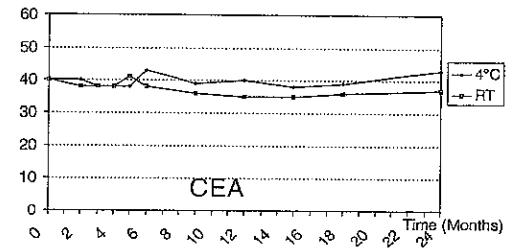
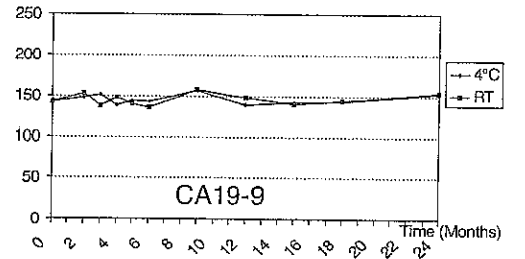
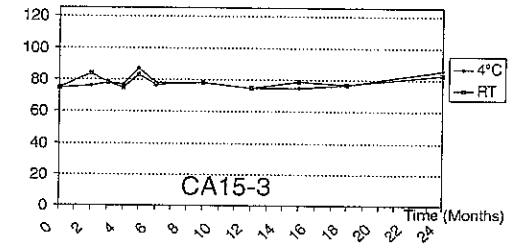
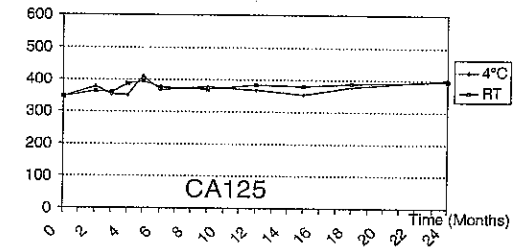


Fig. 1. Stability of some BIOREF tumor markers. BIOREF reference material was stored at room temperature (RT) and +4 °C. After different periods of time the analyte concentration was measured using the Roche ES 600 assay.

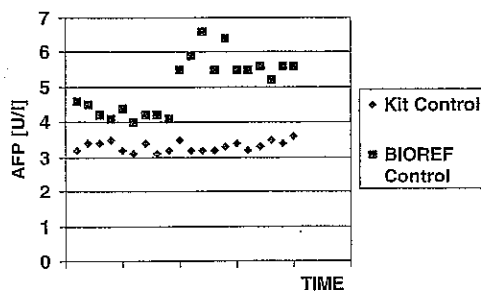


Fig. 2

Example of a shift of measured values due to the change of a test kit lot. AFP routine determinations were done in the author's laboratory using the Roche Cobas Core assay. BIOREF control serum was stored at +4 °C.

reconstituted material in aliquots in a freezer. In many cases these controls consist of an artificial matrix with biochemically treated antigens which can result in discrepant immunoreactive behavior compared to patient sera.

An alternative to the above mentioned sources of tumor marker reference materials are liquid BIOREF controls which contain all relevant tumor markers. They consist of a human serum matrix and tumor derived, native antigens which are not treated by chemical or biochemical procedures. Therefore they are very similar in composition to patient sera. Due to the liquid nature and the high stability of the antigens (see Fig. 1) these controls are very easy to handle and are an ideal tool for the long-term quality control. BIOREF reference materials can be used as a test system independent external control to reveal batch variation of tests [see Fig. 2] and support the user when a change between different test systems has to be performed.

#### REFERENCES

1. Klapdor R. Überlegungen zu einem sinnvollen Einsatz von Tumormarkern. *Onkologie* 1: 627-633, 1995.
2. Birkmayer GJD, Schwarz MK, Klavins JV: Quality assurance of tumor marker assays. *J. Tumor Marker Oncol* 5: 195-202, 1990.
3. Sturgeon C, Dai F, Duffy MJ, Hasholzner U, Klapdor R, Lamerz R, Malbohan I, Martin M, Troonen H, van Dalen A, Zwirner M: Quality requirements and control: EGTM recommendations. *Anticancer Res* 19: 2791-2794, 1999.
4. Mugan K, Carlson IH, Westgard JO: Planning QC procedures for immunoassays. *J Clin Immunoassay* 17: 216-222, 1994.
5. Bieglmayer C, Klapdor R, Kreienberg R, Lüthgens M, Zwirner M: Ergebnisse einer internationalen Ringstudie zur Qualitätssicherung der Bestimmung des tumorassoziierten Antigens CA 125. *Tumor Diagn Ther* 8: 150-155, 1987.
6. Zwirner M, Bieglmayer CH, Klapdor R, Kreienberg R, Lüthgens M. An international proficiency study with the tumor marker CA 125. *Int J Markers* 5: 55-60, 1990.
7. Zwirner M, Risse T, Benz R, Birkmeyer GD, Buchterkirche W, Baum U, et al. Determination of CA 19-9 with various commercial assays. Results of an international proficiency study. In: Klapdor R, editor. *Current tumor diagnosis: Applications, clinical relevance, research, trends*. München, Bern, Wien, San Francisco: Zuckschwerdt, 1994: 481-486.
8. Zwirner M, Bieglmayer C, de Bruijn HW, Crombach G, Ebert G, Kreienberg R, et al. Results of an international proficiency study with the SCC tumor marker. In: Klapdor R, editor. *Recent results in tumor diagnosis and therapy*. München, Bern, Wien, San Francisco: Zuckschwerdt, 1990: 285-298.
9. Culić Z, Archibald BL, Bichler KH, v. Dalen A, Kießing F, Klapdor R, Klocker H, Lüdtker R, Marink J, Mössner E, Obst I, Schmid L, Wagner H, Wieser N, Zwirner M: Longitudinal quality control with tumor marker tests: Results of an international proficiency study with prostate-specific antigen. *Lab Med* 22: 354-360, 1998.
10. Vorstand der Bundesärztekammer: Richtlinie der Bundesärztekammer zur Qualitätssicherung quantitativer laboratoriumsmedizinischer Untersuchungen. *Deutsch Ärztebl* 98: C2188-C2199, 2001.
11. Sonntag O: Matrixeffekte und ihre Bedeutung bei der Verwendung von Kontrollmaterialien im medizinischen Bereich. *J Lab Med* 22: 209-216, 1998.
12. European Parliament and European Council: Directive 98/79/EG of the European Parliament and Council of October 27, 1998, about in-vitro diagnostic medical devices. *Official Journal* No. L331: 1-37, 1998.