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Letter to the Editor

Revision of the Standards for the Assessment of Hormone Receptors in Human Breast Cancer; Report of the Second E.O.R.T.C. Workshop, Held on 16–17 March, 1979, in the Netherlands Cancer Institute*

E.O.R.T.C. Breast Co-operative Group†

In 1972 an E.O.R.T.C. Breast Cancer Group Workshop agreed on standards for the assessment of estrogen receptors in human breast cancer. The results of this workshop were published in 1973 in this Journal [1].

A second workshop on the same subject was held on 16–17 March, 1979 in the Netherlands Cancer Institute (participants*) under the chairmanship of Prof. Dr. P. W. Jungblut.

The goal of this second workshop was to reach agreement on details of the charcoal adsorption technique, to extend the standardization to the progesterone receptor assay and to establish a quality control system for the laboratories of those centres taking part in E.O.R.T.C. Breast Cancer Group Studies‡. In future clinical studies the receptor content may be a factor for patient stratification.

The following report provides notes on changes in and extensions of recommendations published in the first report [1].

EXPERIMENTAL PROCEDURES

1. *Tissue collection and storage*

It is of importance to trim the tumor of adjacent fat (watchglass or Petri dish on crushed ice) and to ensure that the samples taken for histological examination and biochemical analyses are of similar composition. This should preferably be done by a pathologist in the operating theatre. When transportation to the pathologist cannot be avoided, the specimen should be packed in a polyethylene bag and submerged in crushed ice in a suitable container (e.g., Dewar, polyurethane box). Any contact with formalin must be strictly avoided. The tumor should be passed to the laboratory for freezing and storage as quickly as possible after its removal. If it must be shipped to a distant laboratory, the pathologist should cut the tumor into small chunks of 1–2 mm length, pack the tumor pieces in aluminium foil or enclose the pieces in another way to prevent dessication. The tumor pieces should be surrounded by a sufficient amount of dry ice in an insulated box to ensure arrival in the frozen state. Storage at the laboratory where the analysis is performed should be preferably in liquid nitrogen and for not longer than 4 weeks.

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2. *Processing for biochemical analyses*

For full scale evaluation, a minimum of 0.25 g tissue (wet weight) is required. Pulverization in a N_2-chilled mortar or in a 'dismembrator' (Braun, Melsungen, Germany) remains the methods of choice for homogenization. Pulverization with a dismembrator should be performed for 3×1 min with intermittent cooling periods of 5 min in liquid nitrogen. After pulverization the powder tissue is kept frozen until all tumours of one run have been pulverized. Then the homogenate is prepared by adding buffer (4°C) to the pulverized powder and sucking up and down; the use of a vortex mixer is avoided. This way the time between thawing up (by addition of the buffer) and addition of labelled estradiol, is identical for all homogenates.

The extraction buffer is 0.01 M K_2HPO_4/KH_2PO_4, 0.0015 M K_2EDTA, 0.003 M NaN_3, 0.01 M monothioglycerol pH 7.5, 10% v/v glycerol for estrogen receptor and progesterone receptor assay as well. Particle-free extracts are preferred over low-speed supernatants containing mitochondria, lysosomes, microsomes and ribosomes. The tissue/buffer proportion should not exceed 1:8. A minimal protein concentration of 1 mg/ml after addition of charcoal suspension is essential; gelatine can be used as an expander if necessary. Ultracentrifugation should be done maximally for 1 hr.

3. *Labelled steroids*

Minimal purity should be 95%. Tetra- and hexalabelled compounds should be checked weekly, twin-labelled compounds at least monthly. The labelled steroid is diluted with alcohol to a stock solution immediately after arrival (twin-labelled compounds $< 1\ \mu g$/ml, tetra- and hexalabelled compounds to proportionally lower concentrations; storage at −20°C).

Aqueous solutions should be prepared daily. Recommended steroids: for estrogen receptor assay, estradiol; for progesterone receptor assay, R-5020, ORG-2058, D-norgestrel (interference with androgen receptor possible!).

4. *Receptor assay*

The charcoal technique/Scatchard plot procedure is retained as the standard. Steroid concentrations in single point assays should not be lower than 5×10^{-9} M; a parallel incubation with an excess of cold steroid (at least 100-fold) is required in this case. Diethylstilbestrol is given preference over estradiol as a competitor because of its non-binding to SHBG. Incubation is done overnight at 4°C. After addition of the charcoal the mixture is turned overhead for 10 min at 4°C. Mixing with a vortex is not allowed. Triplicate determinations are run for each sample. The quality of charcoal should be such that all free steroid will be removed. Residual counts should be no more than 1% of the added amount. The charcoal suspension should contain 0.05% dextran T70. During handling and incubation the estradiol should be in contact with glass only.

5. *Expression of receptor content*

(*a*) *In extracts:* per mg total protein. The protein content is determined by the method of Bradford [2]. The constituents for this assay are commercially available in the 'Bio-Rad Protein Assay' testkit. As a reference, however, the Kabi protein standard containing (100 g/l) human albumin is used. The protein content is preferably corrected for plasma protein contamination, by albumin assay.

(*b*) *In homogenates (sediments of high speed centrifugation):* per mg DNA using deoxyribose as a standard and the factor 5 for conversion to DNA.

6. *Optional procedures and additional assays*

Every procedure replacing the charcoal technique must be proven equal or better. The analysis of estradiol in the high-speed sediments by radioimmunoassay is highly recommended as a further characteristic.

7. *Provisions for quality control*

Twice yearly, participating laboratories will receive lyophilized calf uterus homogenates from Dr Th. J. Benraad, Nijmegen, The Netherlands. The results will be assessed in Nijmegen.

REFERENCES

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2. M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Analyt. Biochem.* **72,** 248 (1976).