A PARTIALLY AUTOMATED RADIOLIGAND BINDING ASSAY SYSTEM FOR USE IN CLINICAL AND PHARMACEUTICAL RESEARCH

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ABSTRACT

Using a Tecan robotic sample processor and IBM compatible PCs we have developed a flexible, partially automated radioligand binding assay system. It handles pipetting parameters of up to 16 saturation or competition experiments at a time with up to 24 radioligand- or competitor-concentrations in a range over 4 orders of magnitude per experiment. The system provides enough flexibility so that all pipetting parameters including different tube-, rack-sizes, sample volumina and pipetting sequences may be easily adapted to the large variety of experimental requirements in binding assays. It rationalizes and increases assay throughput (up to 70% spare of working time), improves reliance and reproducibility of results. Radioactive exposure is minimized to the time preparing the radioligand working solution and transferring the sample tubes to and from the sample processor. The system has proven effective in various investigations on binding interactions, as well as in clinical studies on receptor expression under physiologic, pathological and therapeutic conditions.

INTRODUCTION

Binding assays with radiolabeled drugs are used to measure and characterize the interactions of ligands with a variety of receptors for hormones, neurotransmitters and drugs. Over the past two decades radio-
Ligand binding techniques have become increasingly important in clinical research and in the pharmaceutical industry as an essential part in drug discovery and evaluation. In clinical investigations receptor expression is studied in tissue samples from healthy, sick, untreated or treated subjects with the aim of correlating changes in their characteristics to the pathophysiology and pharmacotherapy of diseases. In the process of drug discovery the technique allows in vitro targeting of a distinct receptor thought to be involved in the pathophysiology of a given disease which thus may yield important information regarding selectivity, specificity and/or affinity of novel compounds intended for use as therapeutic drugs (1).

A wide range of assays have been developed. Applications in clinical research often involve the study of variations in the expression of various receptors in dynamic situations where receptor properties may alter along a timescale and profile measurements are necessary, for example in drug-response or circadian studies. In order to measure these alterations effectively, it is necessary to take samples for assay at set intervals over a certain time period, e.g. before and after drug application. In most cases this means that many assays have to be performed within a short time for each patient, thus necessitating rapid sampling and assay techniques which generally involves a variety of experimenters working on the same system. In the drug development process many thousands of compounds have to be screened before one can be introduced as new drug.

Valid results may only be obtained under well defined, standardized experimental conditions which in turn may differ largely depending on various experimental protocols. The scatter of data points is a most critical experimental parameter and validity therefore highly depends on the reproducibility of the results which in turn relies upon the accuracy and precision of the assay steps involved. Ligand binding assays are tedious procedures involving a large number of pipetting steps combined with radioactive exposure of the lab personnel. Manual pipetting techniques have considerable potential for dispensing imprecision and error associated with liquid carry over and contamination. In addition, when several workers are involved in a study, the variation in pipetting skills leads to additional error.

There has been considerable interest in the automation of such procedures but because of the variety of assays, it has not previously been
possible to develop a flexible and satisfying system. Using a robotic
csample processor and a personal computer (PC) we have developed a
partially automated radioligand binding assay system which rationalizes
and increases assay throughput, improves reliability and reproducibility of
results, reduces radioactive exposure and frees lab workers from time-
consuming pipetting jobs.

ASSAY SYSTEM

We have designed a software (EBSS = equilibrium binding studies
software) for IBM compatible PCs controlling a Tecan Robotic Sample Pro-
cessor Model 5032 (dual arm system). The system handles pipetting para-
eters of two types of experiments, saturation or competition experiments
(up to 16 single assays at a time). Up to 24 concentrations of a radioligand
or competing compound in a range over 4 orders of magnitude may be de-
fined per experiment. An additive (e.g. antioxidans) may be supplemented
to all samples uniformly. Pipetting may be performed alternatively in 1 of 3
different tube or rack types, respectively, with total sample volumes
between 200 and 2000 ul. Depending on the desired pipetting volumina,
the syringes of the sample processor are adapted to achieve maximal pi-
petting precision (± 2 % of nominal value). The pipetting sequence can be
altered, automatic pipettes may be switched on or off so that certain pipet-
ting steps may be performed by hand or at a later point of time (e.g. tissue
pipette, if samples are prepared for an experiment which is to be started
later by tissue addition).

All parameter inputs are menu controlled. A hardcopy of the
screens with three main input menus defining assay parameters (for the
example described later) are shown in FIG. 1 - 3. Depending on substance-
specific constants (molecular weights, specific activity) and experiment-
specific variables (volumes, concentrations) required reagent-dilutions (ra-
dioligand, competing compounds, additive) and titertube arrangements are
displayed. The pipetting steps in performance are displayed and may be
printed on a hardcopy device together with exact time course informations.

The system provides enough flexibility so that all pipetting param-
ters can easily be adapted to the large variety of experimental requirements
**FIG. 1:** Screen-hardcopy of input menu defining processor configuration, material and liquid handling parameters.

**FIG. 2:** Screen-hardcopy of input menu defining substance- and tissue-specific parameters.
RADIOLIGAND BINDING ASSAY SYSTEM

FIG. 3: Screen-hardcopy of input menu describing one competition experiment. Up to 16 different experiments are possible per run.

in binding assays. All parameters can be saved in so-called method files and loaded bypassing the input functions, they may be copied, deleted or revised and saved anew. Other options allow a parameter-listing or the generation of an ASCII-format file which may be later combined with radioactivity measurements for data analysis.

If separation of bound from unbound radioactivity is performed by centrifugation, the system may be additionally used for removal of the supernatant.

APPLICATIONS

The advantages of the system is demonstrated in the following by a set of competition experiments which were performed in our lab for characterization of binding sites for $^{125}$Iodocyanopindolol ($^{125}$ICYP) in human peripheral mononuclear leucocytes (MNL) (2-5).
Using the sample processor three competition experiments (in triplicates) with 8, 24 and 95 pmol/l $^{125}$ICYP, respectively, and 21 concentrations of isoprenaline in the concentration range of $10^{-10}$ - $10^{-1}$ mol/l were prepared. Total and unspecific binding (in duplicates) were determined in parallel samples with and without $10^{-6}$ mol/l of (-)-timolol. Altogether 70 specimens per experiment (210 altogether) were prepared with varying volumes of buffer, various isoprenaline dilutions, a constant volume of radioligand- (100 ul) and timolol-solution (20 ul) as well as 200 ul of cell-suspension to achieve a total reaction volume of 1000 ul. FIG. 1 - 3 show hardcopies of the input screens for assay-parameter definition for one of these experiments.

Before introduction of the assay system in our lab one person was employed for at least 4 hours for sample preparation for such a set of experiments, while another lab worker was occupied with cell preparation. Using the processor sample preparation could be achieved by a single experimenter parallel to preparation of MNL by density gradient centrifugation. After parameter definition, working time for sample preparation was reduced to arranging the titer tubes (1 ml Biorad$^R$ in microtiterplate-sized racks) and preparing stock solutions for radioligand and competing compounds (isoprenaline, timolol) according to instructions from the program. Automatic pipetting of one experiment took about 10 minutes.

Experiments were started by adding 200 ul pMNL suspension in a concentration of $5 \times 10^6$ cells/ml to each of the 210 specimens. After incubation in a waterbath (2 h, 37°C) the sample-racks were centrifuged at 5000 g for 10 minutes and transferred back to the lab robot. Bound radioactivity was separated from the unbound ligand by removing the supernatant by the machine. Compared to the conventional rapid filtration-method over glass fiber filters this procedure saved material (no filters necessary), time and radioactive exposure of the experimenters.

The radioactive counts were evaluated using a PC. Due to the pipetting precision of the sample robot scatter of data points was in the range of $\pm 4\%$ of the mean value. To determine the binding parameters (binding capacity $B_{max_1}$ and $B_{max_2}$ for high and low affinity binding sites, the respective equilibrium dissociation constants $K_{i_1} / K_{i_2}$ for isoprenaline and $K_{d_1} / K_{d_2}$ for $^{125}$ICYP)(2) data were mathematically fitted to a binding
A summarized comparison of time consumption and exposure to radioactivity with and without use of sample processor and PC is given in TABLE 1. Altogether 70% of working time was saved (84 min compared to 302 min). Radioactive exposure was minimized to the time of transferring the sample racks from the robot to the waterbath, to the centrifuge and back to the robot. After preparing the radioligand working solution no further direct contact with radioactivity was necessary.

The assay system has been applied in several clinical studies (e.g. circadian and drug-response studies) on receptor expression under physiologic, pathological and therapeutic conditions. Results from these investigations have been published elsewhere (6-11). They implied multiple satu-
TABLE 1.
Comparison of time consumption and exposure to radioactivity (- = none, + = low, ++++ = high) with and without use of sample processor and PCs for 3 competition experiments (21 concentrations in triplicates each).

<table>
<thead>
<tr>
<th>TASK</th>
<th>WITHOUT SAMPLE-PROCESSOR AND PC</th>
<th>WITH SAMPLE-PROCESSOR AND PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total time (min)</td>
<td>working radioact. exposure</td>
</tr>
<tr>
<td>cell preparation by density gradient centrifugation</td>
<td>120</td>
<td>30</td>
</tr>
<tr>
<td>inscription of tubes</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>arrangement of tubes in racks</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>calculation of dilutions</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>prepare stock dilutions</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>prepare intermediate dilutions</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>pipetting 60 tubes (radioligand,buffer,displacer,cells)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>transfer to waterbath, incubation</td>
<td>120</td>
<td>5</td>
</tr>
<tr>
<td>transfer to, load centrifuge centrifugation, unload centrifuge</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>separation bound from free radioactivity</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>load radioactivity-counter and counting</td>
<td>125</td>
<td>5</td>
</tr>
<tr>
<td>raw data analysis</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>nonlinear iterativ curve fit</td>
<td>not possible</td>
<td>-</td>
</tr>
<tr>
<td>SUM</td>
<td>637</td>
<td>302</td>
</tr>
</tbody>
</table>

 ration experiments, for example at 4 hour intervals and 30, 60, 120, 180, 240 and 360 minutes after drug application. Up to 14 experiments with 12 radioligand concentrations per experiment had to be performed within 36 hours for each subject (8, 10). Without the lab robot 2 persons were busy for 1 day preparing the assays for such a study (inscription of titer tubes, arranging them in racks, making reagent dilutions, pipetting jobs). By employing the lab robot preparation time was reduced to about 4 h and could be achieved by a single technician parallel to other tasks in the course of the study.
The system has also proven effective in the research department of a major German pharmaceutical company. It is applied there for the screening of novel compounds for possible binding interaction using the option of performing multiple competition experiments (up to 16 per run) with a small number of concentrations (1 to 4) per substance to be investigated. The responsible scientists report that the same number of experiments which previously afforded 3 technicians is performed now by a single person in half of the time.

CONCLUSIONS

Radioligand binding assays are a well established technique in pharmacological research. They are technically simple to perform, but time-consuming and prone to error if performed manually. Valid information is only obtained if conditions are carefully controlled. Using a robotic sample processor and IBM compatible PCs a flexible, partially automated assay system has been developed which rationalizes and increases assay throughput, improves reliance and reproducibility of results and minimizes radioactive exposure of experimenters.

REFERENCES


