Review


# AN OVER REVIEW- HIGH THROUGHPUT SCREENING

**Rajesh Asija, Radheyshyam Kumawat, Sandeep Kataria, Deepak Sharma, Preethi Sagar**

**Maharishi Arvind Institute of Pharmacy, Jaipur**

Submitted on: 24.04.2015 Revised On: 15.05.2015 Accepted on: 19.05.2015

**ABSTRACT:**

High Throughput Screening is one of the newest methods in drug design. It improves the efficiency of drug screening and minimizes the animal testing. The large number of compounds produced by combinatorial chemistry and the possibility of testing many compounds in a short period of time by HTS attracted attention of many workers. Various detection techniques are available. Typical HTS have the capacity to screening up to 10000 compounds per day and ultra HTS can perform 100,000 per day.

**KEYWORDS:** High throughput screening, immortalised cell lines, MTT assay etc.

**Corresponding author : Preethi Sagar**
**Email id:** preethirose001@gmail.com

Indian Research Journal of Pharmacy and Science; 5(2015) 91-99;
Journal home page: https://www.irjps.in

## 1. INTRODUCTION:

High-throughput screening(HTS)is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. Researchers uses data processing systems, robotics, liquid handling systems, control softwares, sensitive detectors in HTS to conduct many of chemical, genetic or pharmacological tests. The results help to provide starting points for drug design and also for understanding the interaction or role of a particular biochemical process in biology.

It is basically a process of screening and assaying huge number of biological modulators and effectors against selected and specific targets. The HTS is used for the screening of genomics, chemistry, and peptide, protein libraries. The goal of this technique is to hasten the drug discovery process by screening the large compound libraries with a speed which may exceed a few thousand compounds. There are many steps are involved in the process of HTS. These should be carried out with care and precision.

HTS helps in drug discovery and also in the development of drug moieties to optimize their activity. Research is also carried out to reduce the drug development costs. Initially the assays were carried out in 96-well plates but with advancement now there are also 1586-well plates available. Typical HTS programs have potentials to screening up to10000 compounds per day, while some laboratories with Ultra High-Throughput Screening (UHTS) has the capacity to perform 100,000 assays per day.

### 1.2. COMMON TERMS USED:

**1.2.1 Assay:** a test system in which biological activity can be detected

**1.2.2 Hit:** control molecule with known chemical structure and activity

**1.2.3 Progressible hit:** a representative of a compound series with activity via acceptable mechanism of action and some limited structure-activity relationship information

**1.2.4 Lead:** a compound with all measured parameterss to progress to a full drug development programme

**1.2.5 Pharmacophore:** minimal structure with essential features for activity

### 1.3. Anatomy of a High Throughput Screening System

The HTS system includes control software, robotic handling systems, and sensor systems, liquid handling systems. Microplates, wells are moved through the system by robotic handling system. After the incubation process the wells are filled with liquid handling systems. The sensors are used to evaluate the samples in the microtiter plate. Control software choreographs the whole process, affirm accuracy within the process and repeatability between processes.

In the past, screening was a slow because of human labor to perform the screening and human observation to measure results. Automation step in the HTS has greatly increase the number of screening along with the maintaining quality. This step reduces the time requirement for drug discovery and human labor.

### 1.4. Types of High Throughput Screening Assay

HTS assays can be divided into two types, namely cell-based assays and biochemical assays. Biochemical assays, mainly enzyme inhibition and receptor-ligand binding assays, are the main types of HTS operations in the pharmaceutical industry. In a biochemical assay, the specific binding or affinity of tested compounds is carried out in homogeneous reactions allows the less variations. The responses tested in biochemical assays cannot precisely represent tissue-specific responses because the activity of a small molecule in a biochemical assay is different from the activity in a cellular context. Therefore, the toxicity testing market is gradually shifting towards in vitro cell-based assays, they provide an early indication of the toxicity characteristics of the drug candidates.

Cell based assays are extensively applied in the majority of compound screening programs performed by the biopharmaceutical industry. These assays have the capacity to distinguish between agonists and antagonists, identify allosteric modulators, and provide direct information on compounds with regards to cell permeability and stability inside cells, and acute

cytotoxicity associated with the compounds. The components of a cell-based HTS assay are cells, devices for culturing the cells, and detection systems for quantification of cells or cellular activities.

### 1.5. CELL SOURCES AND TYPES

Cells used in cell-based assays should be amenable to the assays, faithfully represent the system and express the necessary factors and signaling intermediates. Different types and sources of cells have been used in cell-based assays.

Immortalized cell lines are widely used for drug screening assays because they are easy to grow, reliable and reproducible and cheap. HEK293 cells, derived from human kidney, are an easy. Immortalized cell lines, derived from tissue, have undergone significant mutations and their biological characteristics might be altered in the immortalization process and different from those of the native cells. Primary cells may provide representative responses; however, they have a limited life span in culture and are difficult to grow and transfect. Human cancer cell lines represent the cancer of origin and are widely used for anticancer drug screening in pharmaceutical research. However, they can contain mutations that might affect the experimental results. For instance, the breast cancer cell line MCF-7 lacks a functional caspase-3 gene product. Furthermore, cell-based assays employing primary cells or immortalized and cancer cell lines have been insufficient in developing effective therapeutics for cancers. Although the initial therapyand recovery is successful, but rate to a relapse of the disease is very high.

Table 1: Types of Cell-Based Assays Used in HTS for Drug Screening

| Assay type | Mechanism or method | Example |
|---|---|---|
| Second messenger | signal transduction following activation of cell-surface receptors | Using fluorescent molecules that respond to changes in intracellular Ca2+ concentration, membrane potential, pH, etc. to assay receptor stimulation and ion channel activation |
| Reporter gene | cellular responses at the transcription/translation level | Quantification of G-protein coupled receptor (GPCR) internalization using a GPCR-green fluorescent protein hybrid |
| Cytotoxicity | the overall cell growth or death in response to external stimuli or stress | Virus-induced cytopathic effects on cell proliferation monitored by following the reduction of tetrazolium salt to formazan quantified by absorbance at 410 |

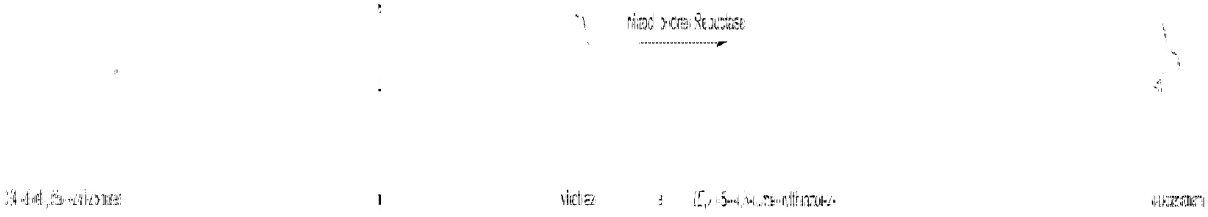
### 1.5.1. STEPS:

#### 1.5.1.1 Assay plate preparation

**microtiter plate:** A small container, that contain some series of small open divots called *wells*. Most of the wells contain experimentally useful matter. This could be an aqueous solution of dimethyl sulfoxide(DMSO) and some other chemical compound, the latter of which differs for each well across the plate. It could also contain cells or enzymes of some type.

Assay plates are used in the experiment. An assay plate is created by pipetting a small amount of liquid (often measured in nanoliters) from the wells of a stock plate to the corresponding wells of a completely empty plate.

**MTT reaction:**



#### 1.5.1.2 MTT Assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinatedehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then

solubilised with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilised

formazan reagent is measured spectrophotometrically. Since reduction of MTT can only

occur in metabolically active cells the level of activity is a measure of the viability of the cells.

### 1.5.2. Reaction Observation:

To prepare for an assay, the researcher fills each well of the plate with some logical entity that he wishes to conduct the experiment upon, such as a protein, cells, or an embryo. After incubation time has passed to allow the biological matter to absorb, bind to, or otherwise react with the compounds in the wells, measurements are taken manually or by a machine. Manual measurements are necessary for microscopic changes or for embryonic developments caused by well's compound. A specialized automated analysis machine can also be used to run a number of experiments on the wells. A high-capacity analysis machine can also be used to measure dozens of plates generating the thousands of experimental data points very quickly.

Depending on the results of this first assay, the researcher can perform follow up assays within the same screen by "cherrypicking" liquid from the source wells that gave interesting results (known as "hits") into new assay plates, and then re-running the experiment to collect further data on this narrowed set, confirming and refining observations.

### 1.5.3 Automation Systems

Automation is an important element in HTS. An integrated robot system consisting of many robots which transports assay-microplates from station to station for several steps. The system involves in several steps like preparation, incubatation, and analysis of many plates. HTS robots that can test up to 100,000 compounds per day currently exist.

### 1.5.4 Experimental design and data analysis

The most fundamental challenges in HTS experiments is to gather the biochemical significance from the data, which depends on the development and adoption of appropriate experimental designs and analytic methods for both quality control and hit selection.

## 1.6 QUALITY CONTROL

High-quality HTS assays are critical in HTS experiments. The development of high-quality HTS assays requires the integration of both experimental and computational approaches for quality control (QC). Three important means of QC are (i) good plate design, (ii) the selection of effective positive and negative chemical/biological controls, and (iii) the development of effective QC metrics to measure the degree of differentiation so that assays with inferior data quality can be identified. A good plate design helps to identify systematic errors (especially those linked with well position) and determine what normalization should be used to remove/reduce the impact of systematic errors on both QC and hit selection.

Effective analytic QC methods are very important for maintaining the quality of the assays. In a HTS experiment, a clear distinction between a positive control and a negative control is an index for good quality. Quality-assessment measure have been proposed to measure the degree of differentiation between a positive control and a negative reference. Signal-to-background ratio, signal-to-noise ratio, signal window, assay variability ratio, Z-factor have been adopted to evaluate data quality. Strictly standardized mean difference (SSMD) has recently been proposed for assessing data quality in HTS assays.

## 1.7 HIT SELECTION

A compound with a desired size of effects in an HTS screen is called a hit and the process of selecting hits is called hit selection. The analytic methods which are used for hit selection without replicates differ from those with replicates. The z-score method is suitable for screens without replicates whereas the t-statistic is suitable for screens with replicates. The calculation of SSMD for screens without replicates also differs from that for screens with replicates.

The z-score method or SSMD, which can capture data variability based on an assumption that every compound has the same variability as a negative reference in the screens. However, outliers are common in HTS experiments, and methods such as z-score are sensitive to outliers and can be problematic. In a screen with replicates, can directly estimate variability for each compound; as a consequence, we should use SSMD or t-statistic that does not rely on the strong assumption that the z-score and z*-score. One thing with the use of t-statistic and associated p-values is that they are affected by both sample size and effect size. Both will come from testing for no mean difference, and thus are not designed to measure the size of compound effects. For hit selection, the major interest is the size of effect in a tested compound. SSMD directly assesses the size of effects. SSMD has also been shown to be better than other commonly used effect sizes .The population value of SSMD is comparable across experiments and, thus, we can use the same cut off for the population value of SSMD to measure the size of compound effects.

## LITERATURE REVIEW

**1. Ru Zang Ding Li, I-Ching Tang, Jufang Wang and Shang-Tian Yang (2012)** improve drug screening efficacy and minimize animal testing, recent efforts have been dedicated to developing cell-based high throughput screening (HTS) platforms that can provide more relevant in vivo biological information than biochemical assays and thus reduce the number of animal tests and accelerate the drug discovery process. Today, cell-based assays are used in more than half of all high-throughput drug screenings for target validation and ADMET (absorption, distribution, metabolism, elimination and toxicity) in the early stage of drug discovery. In this review, we discuss the uses of different types of cells and cell culture systems, including 2D, 3D and perfusion cell cultures, in cell-based HTS for drug discovery. Optical and electrochemical methods for online, non- invasive detection and quantification of cells or cellular activities are discussed. Recent progresses and applications of 3D cultures and microfluidic systems for cell-based HTS are also discussed, followed with several successful examples of using cell-based HTS in commercial development of new drugs.[11]

**2. Paweł Szymański, Magdalena Markowicz and Elżbieta Mikiciuk-Olasik (2012)** High-throughput screening (HTS) is one of the newest techniques used in drug design and may be applied in biological and chemical sciences. This method, due to utilization of robots, detectors and software that regulate the whole process, enables a series of analyses of chemical compounds to be conducted in a short time and the affinity of biological structures which is often related to toxicity to be defined. Since 2008 we have implemented the automation of this technique and as a consequence, the possibility to examine 100,000 compounds per day.[12]

**3. Wenge Zhu, Chrissie Y. Lee1, Ronald L. Johnson, Jennifer Wichterman (2011)** developed high-throughput screening that measures DNA replication in excess of four genomic equivalents in the nuclei of intact cells and indexes cell proliferation. This assay was validated by screening a library of 1,280 bioactive molecules on both normal and tumor-derived cells where it proved more sensitive than current methods for detecting excess DNA replication. This screen identified known inducers of excess DNA replication, such as inhibitors of microtubule dynamics, and novel compounds that induced excess DNA replication in both normal and cancer cells. In addition, two compounds were identified that induced excess DNA replication selectively in cancer cells and one that induced endocycles selectively in cancer cells. Thus, this assay provides a new approach to the discovery of compounds useful for investigating the regulation of genome duplication and for the treatment of cancer.[13]

**4. Martis E.A, Radhakrishnan R., Badve R.R, (2011)** The mechanism-based approach which corresponds to the target-based approach screens for compounds with a specific mode of action. The highly effective nature of high-throughput screening (HTS) for identification of highly target specific compounds is attributed to its precise focus on single mechanism. This logical development of receptor technology is closely connected with the changes in strategy of chemical synthesis. The vast number of compounds produced by combinatorial chemistry and the possibility of testing many compounds, including natural products, in a short period of time by HTS attracted attention of many workers. Various detection techniques like fluorescence resonance energy transfer (FRET),

Homogeneous time resolved fluorescence (HTRF), etc are available, and the screening of more than 100,000 samples per day is possible. With the introduction of robotics, automation and miniaturization techniques, it became feasible to screen 50,000 compounds a day with complex work-stations. High-throughput screening methods are also used to characterize metabolic and pharmacokinetic data about new drugs.[14]

**5. Johan van Meerloo, Gertjanj. L. Kaspers, Jacqueline Cloos (2011)** The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. In this chapter the protocol of the assay is described including important considerations relevant for each step of the assay as well as its limitations and possible applications.[15]

**6. Zhengrong Zhu and John Cuozzo (2009)** High-throughput affinity-based technologies are rapidly growing in use as primary screening methods in drug discovery. In this review, their principles and applications are described and their impact on small-molecule drug discovery is evaluated. In general, these technologies can be divided into 2 groups: those that detect binding interactions by measuring changes to the protein target and those that detect bound compounds. Technologies detecting binding interactions by focusing on the protein have limited throughput but can reveal mechanistic information about the binding interaction; technologies detecting bound compounds have very high throughput, some even significantly higher than current high-throughput screening tech- nologies, but offer limited information about the binding interaction. In addition, the appropriate use of affinity-based tech-nologies is discussed.[16]

**7. Lorenz M. Mayr and Peter Fuerst (2008)** High-throughput screening (HTS) is a well-established process in lead discovery for pharma and biotech companies and is now also being set up for basic and applied research in academia and some research hospitals. Since its first advent in the early to mid- 1990s,the field of HTS has seen not only a continuous change in technology and processes but also an adaptation to various needs in lead discovery. HTS has now evolved into a quite mature discipline of modern drug discovery. Whereas in previous years,much emphasis has been put toward a steady increase in capacity ("quantitative increase") via various strategies in the fields of automa- tion and miniaturization, the past years have seen a steady shift toward higher content and quality ("quality increase") for these biological test systems. Today, many experts in the field see HTS at the crossroads with the need to decide either toward further increase in throughput or more focus toward relevance of biological data. In this article, the authors describe the development of HTS over the past decade and point out their own ideas for future directions of HTS in biomedical research. They predict that the trend toward further miniaturization will slow down with the implementation of 384-well, 1536-well, and 384 low-volume-well plates.[17]

**8. Eric Jones, Sam Michael, G. Sitta Sittampalam (2007)** the instrument type is described along with the general principles of operation to familiarize readers considering equipping drug discovery laboratories principally directed to new investigators. The descriptions are introductory and detailed information on installation and applications should be obtained

from instrument vendors and experienced drug discovery scientists and engineers.[7]

**9. DA Pereira and JA Williams (2007)** found the evolution of high throughput screening (HTS) through the experience of an individual pharmaceutical company, revealing some of the mysteries of the early stages of drug discovery to the wider pharmacology audience. HTS in this company (Pfizer, Groton, USA) had its origin in natural products screening in 1986, by substituting fermentation broths with dimethyl sulphoxide solutions of synthetic compounds, using 96-well plates and reduced assay volumes of 50-100ml. A nominal 30mM source compound concentration provided high mM assay concentrations. Starting at 800 compounds each week, the process reached a steady state of 7200 compounds per week by 1989. Screening in the Applied Biotechnology and Screening Group was centralized with screens operating in lock-step to maximize efficiency. Initial screens were full files run in triplicate. Autoradiography and image analysis were introduced for 125I receptor ligand screens. Reverse transcriptase (RT) coupled with quantitative PCR and multiplexing addressed several targets in a single assay. By 1992 HTS produced 'hits' as starting matter for approximately 40% of the Discovery portfolio. In 1995, the HTS methodology was expanded to include ADMET targets.[9]

**10. Sandra Fox, Shauna Farr - Jones, Lynne Sopchak, Amy Boggs, (2006)** High-throughput screening (HTS) has become an important part of drug discovery at most pharmaceutical and many biotech- nology companies worldwide, and use of HTS technologies is expanding into new areas. Target validation, assay development, secondary screening,ADME/Tox, and lead optimization are among the areas in which there is an increasing use of HTS tech- nologies. It is becoming fully integrated within drug discovery, both upstream and downstream, which includes increasing use of cell-based assays and high-content screening (HCS) technologies to achieve more physiologically relevant results and to find higher quality leads. In addition, HTS laboratories are continually evaluating new technologies as they struggle to increase their success rate for finding drug candidates. The material in this article is based on a 900-page HTS industry report involving 54 HTS directors representing 58 HTS laboratories and 34 suppliers.[20]

**Conclusion:** The HTS field continues to dynamic and extremely competitive one. There is a need to increase the throughput of drug-discovery screening operations while reducing development and operating costs is continuing to drive the development of homogeneous, fluorescence-based assays in miniaturized formats. The use of 384-well and higher density plates and commercially available plate-handling robotics has made HTS a bottom line, and has allowed to achieve ultra-high throughput rates in more than 100,000 samples per day. The system involves in several steps like preparation, incubatation, and analysis of many plates. As the density of plate increases the volume of sample required for the assay is decreased drastically, as a result the assay of expensive drugs can be carried out at lower cost, which compensates the initial setup cost. Manual measurements are used for microscopic changes or for embryonic developments caused by well's compound. A high-capacity analysis machine is used to measure dozens of plates generating the thousands of experimental data points very quickly.

**References**

1 Ru Zang, Ding Li, Ching Tang, Jufang Wang and Shang-Tian Yang "Cell-Based Assays in High-Throughput Screening for Drug Discovery" International Journal of Biotechnology for Wellness Industries, (2012): 1, 31-51

2 Paweł Szymański, Magdalena Markowicz and Elżbieta Mikiciuk-Olasik "Adaptation of High-Throughput Screening in Drug Discovery—Toxicological Screening Tests" Int. J. Mol. Sci. (2012): 13, 427-452

3 Wenge Zhu, Chrissie Y. Lee1, Ronald L. Johnson, Jennifer Wichterman "An Image-Based, High-Throughput Screening Assay for Molecules that Induce Excess DNA Replication in Human Cancer Cells" Mol Cancer Res, (2011): 9(3), 294–310.

4 Martis E.A., Radhakrishnan R., Badve R.R, High-Throughput Screening: The Hits and Leads of Drug Discovery- An Overview, Journal of Applied Pharmaceutical Science 01 (01); 2011: 02-10

5 Johan Van Meerloo, Gertjanj. L. Kaspers, Jacqueline Cloos " Cancer Cell Culture" 10.1007/978-1-61779-080-5_20 2011 237-245

6 Zhengrong Zhu and John Cuozzo, High-Throughput Affinity-Based Technologies for Small-Molecule Drug Discovery, Journal of Biomolecular Screening 2009:1157-1164

7 Lorenz M. Mayr and Peter Fuerst, The Future of High-Throughput Screening, (Journal of Biomolecular Screening 2008:443-448

8 Eric Jones, Sam Michael, G. Sitta Sittampalam "Basics of Assay Equipment and Instrumentation for High Throughput Screening" Assay guidance manual

9 Naoki Maeda, Yasuo Kokai, Seiji Ohtani, Hiroeki Sahara, Takahiko Hada "Anti-Tumor Effects of the Glycolipids Fraction from Spinach which Inhibited DNA Polymerase Activity" Nutrition and Cancer, (2007): 57(2), 216-223

10 Sandra Fox, Shauna Farr - Jones, Lynne Sopchak, Amy Boggs, High-Throughput Screening: Update on Practices and Success, Journal of Biomolecular Screening 2006:864-869

11 DA Pereira and JA Williams "Origin and evolution of high throughput screening" British Journal of Pharmacology, (2007): 152, 53–61


Conflict of Interest Reported: Nil; Source of Funding: None Reported