**Review Article** **CODEN: AJPAD7** **ISSN: 2321 - 0923**

**Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry**

**Journal home page: www.ajpamc.com**

https://doi.org/10.36673/AJPAMC.2025.v13.i03.A07

# ULTRA HIGH THROUGHPUT SCREENING METHODS

**N. D. Nizamuddin*[1] and V. Bhavana[1]**

[1*]Dr. K.V. Subba Reddy Institute of Pharmacy, Dupadu, Kurnool, Andhra Pradesh, India.

**ABSTRACT**

Ultra-High Throughput Screening (uHTS) is a significant advancement in drug discovery, allowing rapid evaluation of extensive chemical libraries with millions of compounds. It enhances traditional high-throughput screening (HTS) through advanced robotics, miniaturized assays and sophisticated data analytics, leading to faster identification of bioactive molecules. Operating in multi-well formats, uHTS reduces reagent use while maintaining high sensitivity. Key components include automated liquid handling, real-time data acquisition and optimized assay strategies, with detection techniques like FRET and high-content imaging. Additionally, machine learning aids in hit identification and predictive modelling. uHTS is also applied in functional genomics and synthetic biology, though it faces challenges like data overload and assay interference. Ongoing technological advancements are set to enhance uHTS capabilities further, solidifying its role in discovering new therapeutics and understanding complex biological systems.

**KEYWORDS**

High-Throughout screening (HTS), Cell-based assay, Biochemical assay, Screening, Technologies and Assay.

**Author for Correspondence:**

N. D. Nizamuddin,

Dr.K.V. Subba Reddy Institute of Pharmacy,

Dupadu, Kurnool, Andhra Pradesh, India.

**Email:** drnizamuddin.chemistry@gmail.com

## INTRODUCTION

The development of pharmaceutical drugs involves a complex process that combines scientific research and advanced technologies. While the perception is that scientists directly create medications from research, in reality, robotic systems are employed by pharmaceutical companies to test millions of compounds for potential efficacy. The journey from initial discovery to an approved drug is fraught with challenges, with a success rate of less than 1 in 250, and it takes an average of 15 years and over $800 million to develop a new drug. To streamline this process, techniques like High Throughput Screening (HTS) have emerged, enabling the screening of

numerous biological effectors to identify therapeutic targets efficiently. HTS allows researchers to identify small molecule modulators in drug development, particularly in areas such as central nervous system (CNS) drug discovery, which involves receptor engagement, hit identification, lead identification, and optimization of drug leads[1].

**Definition**

High-throughput screening (HTS) is a method commonly utilized in drug discovery and related fields such as biology, materials science, and chemistry. It employs robotics, data processing software, liquid handling devices, and sensitive detectors to enable researchers to rapidly conduct millions of tests across chemical, genetic, or pharmacological domains. This approach aids in identifying active compounds, antibodies, or genes that influence specific bio molecular pathways, laying the groundwork for drug design and enhancing the understanding of interactions at specific locations[2].

## HTS PLATFORMS AND TECHNOLOGIES ATOMATION, LIQUID HANDLING, DETECTION TECHNOLOGIES

**Automation**

Advancements in automation and miniaturization of in vitro assays in 384-well and 1536-well formats have enhanced high-throughput screening (HTS) processes, allowing for the testing of hundreds of thousands of compounds daily. These improvements in data analytics, visualization, and informatics provide a comprehensive view of potential drug candidates before allocating further scientific resources. This section reviews various automation, liquid handling, and detection platforms used in screening labs, which typically operate in three automation modes: batch, semi-automated, and integrated. Batch mode involves the use of plate stackers and is generally operated by scientists who manually transfer plates for processing during screening assays[3].

**Plate storage**

There are two main types of plate storage systems: shelf-based systems and stackers. Shelf-based systems include (1) open shelved racks, which provide standard ambient conditions and (2) incubators that maintain controlled temperature, humidity, and gas environments. Each type holds microplates on individual shelves, ensuring consistent exposure to temperature and humidity, which is crucial for minimizing assay variability. Examples of integrated robotic systems for these applications include the High Res Bio solutions Modular System and a custom Tselios-based platform for radioactive assays.

**Robotic arms**

The primary purpose of robotic arms and grippers in screening systems is to move consumables and reagents across assay steps, ensuring consistency throughout the assay run. Common arm types include simple plate movers, which transfer microplates with built-in controllers, and limited access arms, which are more complex with 2-4 degrees of freedom and can interact with stackers and peripherals. Examples include Hudson's Plate Crane and Perkin Elmer's Twister II[4].

**Liquid handling**

Within screening workflows, liquid transfers are a critical component, often being one of the main contributors to assay variability. There are several frequently used dispenser types representing a variety of dispensing mechanisms. Traditionally, tip-based dispenser types with air and positive displacement dispensing mechanisms have been most commonly used in screening. In more recent years, non-touch dispensing types have gained in popularity, especially acoustic Dispensing. Shows examples of common liquid handling types and mechanisms found in Screening[5].

**CCD- based detectors**

High-density plate format screening, including 1536 wells, is made possible by CCD based readers because to their quick detection speeds and decreased well-to-well variability. Fluorescence, luminescence, absorbance and radioactivity are supported by different imagers (for instance, Mesa scale Discovery's SECTOR Imager 6000 and PerkinElmer's View Lux). These systems' features include cooled CCDs to increase sensitivity, coupled telocentric lenses to reduce parallax, longer

exposure times in low light detection assays because the results are results both visually and numerically. Dust interference, the requirement for several raw data modifications (such as parallax, flat field, shading, pixel binning and vignetting) and the upkeep of ultra-low temperature Cameras.

Ex: employed in imaging-based plate readers including choose for whole plate kinetics imaging allowing for spatial resolution and capturing images of entire wells are plates.

**Whole plate kinetic imaging**

Plate readers that allow for fluorescence and luminescent-based kinetic measurements in 96-, 384- and 1536-well formats include the Hamamatsu FDSS7000EXTM and the Molecular Devices FLIPR Tetra. Typical uses for these readers include measuring intracellular calcium, supporting membrane potential tests, enabling transporter assays, and facilitating repeated measurement cardio toxicity assays. They employ cooled CCD detectors

**PMT –based detectors**

The majority of PMT-based readers employ a white light source, like a laser, xenon flash lamp, or tungsten lamp, to increase sensitivity. LEDs have been employed as a light source with a particular wavelength in more recent times. Another way to categorise PMT readers is by the method used to calculate excitation and emission wavelengths: filter-based and monochromatic based. A comparison of the two choices is presented in Filter-based detectors are more common in screening labs because of their faster ability to switch between two wavelengths, higher sensitivity, reduced total cost, and enhanced light transmission efficiency. Monochromatic-based devices are commonly used in mechanism-of-action and assay development labs where scanning a range of wavelengths is a crucial capability. There are examples of various PMT-based microplate reader types and their features displayed. (32)

Ex: used in fluorescence and luminescence plate readers for high sensitivity detection of light signals.

## ASSAY FORMATS IN HTS

**Assay development and assay technologies**

The assay, or "screen," is a crucial element of High-Throughput Screening (HTS), distinguished by its statistical validation. An assay determines biological function, while a screen is a validated assay that meets reliability and reproducibility standards. The rigor and criteria for assays vary, with relevant assays accurately predicting a compound's effect in disease models. The biological mechanisms used for monitoring these effects often involve technologies revealing biological targets, which are critical to HTS discussions. Targets are typically specific proteins linked to disease states, with advanced screens incorporating tissue or cellular systems to explore multitarget interactions, signaling cascades, or unknown targets. A validated target signifies a clear reflection of the disease.

**Biochemical assays**

Using a purified target protein of interest, biochemical screening quantifies ligand binding or enzymatic activity inhibition *in vitro* the molecule being studied displaces a known ligand or substrate in these experiments, which are often carried out in a competition format. Usually, these tests are carried out in 384-well plates, which offer a good balance between throughput, screening quantities (20–50µL) and the price of more expensive screening Using purified target proteins, biochemical screening evaluates ligand binding or enzymatic inhibition in vitro, often utilizing competition formats in 384-well plates with optical readout techniques like absorbance, fluorescence, or luminescence. The RNA binding protein TDP-43's activity was assessed, revealing its role in neurodegenerative diseases (NDDs) such as Parkinson's and Alzheimer's. A significant study screened approximately 300,000 chemicals for their influence on tau protein assembly, crucial in Alzheimer's pathology due to toxic tau oligomer formation. High-throughput screening assays, employing techniques like tholepin T fluorescence, demonstrated that Aminothienopyridazine (ATPZs) can inhibit tau fibrillation while preserving tau's ability to stabilize microtubules. Despite the rise of

lab-on-a-chip technology for cost and space efficiency, challenges such as lengthy processing times and potential robotic errors persist. Biochemical tests effectively pinpoint hits targeting known molecules but reveal that understanding their molecular mechanisms is often costly and time-intensive, with inconsistent therapeutic potentials depending on disease context confirmation[6].

**Cell based assays**

Cell-based assays for HTS can be classified under following classes: Second messenger assays: It monitors signal transduction from activated cell-surface receptors. Second messenger assays typically measure fast, transient fluorescent signals that occur in matter of seconds or Milliseconds. Many fluorescent molecules are known to respond to changes in intracellular Calcium ion concentration, membrane potential and various other parameters, hence they are Used in development of second messenger assays for receptor stimulation and ion-channel Activation. The development of hydrophobic voltage-sensitive probes and FRET compatible Microplate instrumentation has helped the advancement of the screening technique for ion channel drug discovery. Reporter gene assays: It monitors cellular responses At transcription/translation level. It indicates the presence or absence of a gene product that in turn Reflects changes in a signal transduction pathway. The quantification of the reporter is usually carried out by biochemical methods viz by measuring the enzymatic activity. Plasmids are typical reporter genes employed. An entirely *in vitro* study was carried out by Suang Rungpragayphan *et al*. For generation and screening of combinatorial protein library in assay Format. This studied employed virtues of polymerase chain reaction (PCR) and in vitro coupled Reporter gene assay. Cell proliferation assays: It monitors the overall growth/no growth responses of the cell to external stimuli. These are quick and easy to be employed for automation.

**Homogeneous assay**

The unique characteristics of the analyse and its surroundings form the basis for measurements in homogeneous assays. These assays can utilize reagents in a one-step method, combining routine procedures such as reading, incubation, and fluid addition. For high-throughput screening (HTS), they can also integrate other detection methods like radiometric and fluorescence. A key advantage of homogeneous assays is their simplicity, which reduces costs and robotic complexity for automation. However, measurements may experience interference due to other assay components present, affecting the signal-to-background ratio[7].

**Heterogeneous assay**

Additional procedures such as centrifugation and filtration are used in heterogeneous assays to separate the component or components to be analysed from the remaining components that could obstruct the experiment. The high signal to background ratio is a result of this. Higher stairs make it more difficult. When a high signal to background ratio is needed or a homogeneous assay fails, heterogeneous assays are typically conducted[8].

**Geno toxicity assays**

Genetic toxicology is the scientific discipline the aim of which is to establish the effects of chemical, physical and biological agents on the heredity of living organisms. For measurement of Geno toxicity of chemicals the use of the Ames bacterial reverse mutation test lymphoma to gene mutation assay (a negative selection for loss of the functional thymidine kinase gene), and the micronucleus clastogenicity assay are employed. The Ames test, the simplest and quickest of the existing Geno toxicity assays, is capable of detecting point mutations and frame shift mutations. However, it does not detect chromosomal rearrangements or double strand breaks. In the micronucleus assay double strand breaks contribute to formation of chromosomal fragments that are not attached to microtubules during metaphase, and are not pulled to opposite poles before cell division. These chromosome fragments migrate outside the normal nucleus and can be observed microscopically as micronuclei. This assay is prone to false positive results which occur when an undamaged but lagging chromosome forms a micronucleus and false negative results which are caused because the

micronucleus assay detects only double strand breaks Methods listed above possess several drawbacks, such as high costs, low specificity and sensitivity. Furthermore, these tests do not allow the screening of a large number of compounds[9].

**Microfluidics**

Microfluidics is another technology that is new to HTS but is becoming widely applied. This technique utilizes submillimetre channels etched or cast into a solid substrate such as plastic, glass or quartz Fluid flow through these channels can be precisely controlled by pressure or voltage gradients. It is an example of a technology that muddies the simple distinction between homogenous and heterogeneous techniques because it allows many operations to be performed on a single chip including separation technologies the most common of these is the application of capillary electrophoresis to separate charged proteins. Therefore, microfluidics is most commonly used for enzymatic assays although it has also been utilized for cellular screens (82, 83). The combination of precise control in mixing and reaction with the removal pipetting errors also makes microfluidics much more precise than standard microplate based techniques. Microfluidics assay procedures typically involve the manipulation of very small volumes of fluids in micro-scale channels to perform.

## CHALLENGES AND LIMITATIONS OF ULTRA HIGH THROUGHPUT SCRRENING METHODS

**High Cost and Infrastructure Demands**

Explanation: uHTS systems require significant investment in automation equipment, compound libraries, robotic liquid handling systems, and data analysis software.

Limitation: Smaller research institutions or start-ups may not afford the capital investment.

**Data Quality and False Positives/Negatives**

Explanation: The high volume of data increases the chances of false positives (inactive compounds that appear active) and false negatives (active compounds that appear inactive).

Limitation: Requires extensive follow-up validation, which can slow down the discovery pipeline.

**Complexity of Biological Targets**

Explanation: Many uHTS assays focus on simple biochemical targets (e.g., enzyme inhibition), which may not reflect complex cellular or physiological responses.

Limitation: Hits identified may not be effective *in vivo* due to lack of context.

**Compound Aggregation and Interference**

Explanation: Some compounds form aggregates or interfere non-specifically with assay readouts (e.g., fluorescence interference).

Limitation: Leads to art factual results that require counter-screening.

**Limited Assay Types**

Explanation: Not all biological assays are amenable to high-throughput formats-especially phenotypic or cell-based assays involving complex signalling or morphology.

Limitation: Limits the biological relevance of some screens.

**Library Quality and Diversity**

Explanation: The effectiveness of uHTS depends on the diversity and quality of the compound library.

Limitation: Redundant or poorly diverse libraries reduce the chance of novel hits.

**Applications**

Although developed for drug discovery, HTS is now widely used in many industries and Academia. It has also broadened within each. In Academia HTS is being applied to discovery Chemical probes that can be used in basic research. The US National Institutes of Health (NIH) Began a program in 2004 to create a series of interconnected screening centres as part of its Molecular Libraries Screening Initiative In the industries where it is already established, HTS techniques are now being used to perform more complex assays and large scale titrations. Often the latter are the next step in the process of developing a molecule such as selectivity testing where all the confirmed hits may be tested against a fixed panel of assays to identify[10].


**Figure No.1: Examples of integrated robotic systems**

## CONCLUSION

Ultra-high throughput screening (uHTS) has transformed drug discovery and biotechnology by facilitating rapid evaluation of millions of compounds against biological targets. Through advanced automation, miniaturization and data analytics, uHTS accelerates lead compound identification while minimizing costs and resource use. Despite challenges like data management complexity and high initial investments, technological advancements, including AI-driven analysis, microfluidics and next-gen sequencing, improve its capabilities. uHTS is essential in modern high-efficiency screening strategies, playing a crucial role in pharmaceutical research and personalized medicine.

## ACKNOWLEDGMENT

The authors wish to express their sincere gratitude to Dr. K.V. Subbareddy Institute of Pharmacy, Dupadu, Kurnool, Andhra Pradesh, India for providing necessary facilities to carry out this review work.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## BIBLIOGRAPHY

1. Thomford N E, Senthebane D A, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. Natural products for drug discovery in the 21st Century: Innovations for novel drug discovery, *Int. J. Mol. Sci*, 19(6), 2018, 1578.
2. Yicheng Zhao, Jiyun Zhang, Junsheng Luo, Olga Kasian, Stefan Langner, Christian Kupfer, Bowen Liu, Yu Zhong. A bilayer conducting polymer structure for planar perovskite solar cells with over 1,400 hours operational stability at elevated temperatures, *Nature Energy*, 7(2), 2021, 144-152.
3. Macarron R, Banks M N, Bojanic D, Burns D J, Cirovic D A, Garyantes T, Green D V S, Hertzberg R P, Janzen W P, Paslay J W, Schopfer U, Sittampalam G S. Impact of high-throughput screening in biomedical research, *Nat. Rev. Drug Discov*, 10(3), 2011, 188-195.

4. High res biosolutions announces a cell, the most advanced bench top laboratory automation system ever, *High Res Biosolutions A Cell*, 2012.
5. Zheng W, Chen C. Screening automation, A practical guide to assay developmentand high-throughput screening in drug discovery, *CRC Press, Boco Raton, FL, USA,* 2010, 184-185.
6. Tau-directed drug discovery for Alzheimer'sdisease and related tauopathies: A focus on tau assembly inhibitors, *Exp. Neurol*, 223(2), 2010, 304-310.
7. Mukesh D. Drug design basics and application, Tata Macgraw Hill education private limited development of screening assays for drug discovery, *McGraw Hill Education*, 2011, 57.
8. Assay Development: Fundamentals and Practices, *Ge Wu John Wiley Inc Chapter 4 Separation Based Techniques in Bioassays*, 2012, 105.
9. Kirkland D, Aardema M, Henderson L, Muller L. Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogen and non-carcinogens I. Sensitivity, specificity and relative predictivity, *Mutat. Res*, 584(1-2), 2005, 251-256.
10. Kaiser J. NIH gears up for chemical genomics, *Science*, 304(5678), 2004, 1728.

**Please cite this article in press as:** Nizamuddin N D and Bhavana V. Ultra high throughput screening methods, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 13(3), 2025, 45-51.