

HIGH THROUGHPUT SCREENING (HTS)

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HIGH THROUGHPUT SCREENING (HTS) is identification of one or more positive candidates extracted from a pool of possible candidates based on specific criteria.

LEAD compound is a representative of a compound series with sufficient potential (as measured by potency, selectivity, pharmacokinetic, physicochemical properties, toxicity and novelty) to progress to a full drug development programme.

- ❖ It is a **drug-discovery process** widely used in the pharmaceutical industry.
- ❖ It allows automation to quickly assay the biological or biochemical activity of a large no of compounds.
- ❖ HTS is process by which large nos. of compounds are rapidly tested for their ability to modify the properties of a selected biological target.

- ❖ It is a useful for discovering ligands for receptors, enzymes, ion-channels or other pharmacological targets, or pharmacologically profiling a cellular or biochemical pathway of interest.

Screening Collection

Screening libraries:

(a) Diversity Libraries

(b) Focused Libraries :

Ligand-Based Approaches

Structure-Based Approaches

■ Compound acquisition Purchasing commercially available

LIBRARIES

- ❖ **siRNA libraries:** providing the best available RNA interference technology with maximum flexibility The mouse whole genome contain about 16872 gene targets and the Human whole-genome contains 18120 gene targets.
- ❖ **microRNA libraries:** Human libraries of synthetic microRNA mimics and microRNA inhibitors.
- ❖ **Small Compound libraries:** A library of 640 FDA approved drugs (Screen-Well FDA Approved Drug Library, Enzo Life

Steps in HTS

❖ 1 st stage screening

- Test optical clarity, abrasion resistance, and adhesion
- Eliminates ~ 90% of samples

❖ 2 nd stage screening

- Test weather ability, integrity, gloss, and surface smoothness
- ~10% of the samples

DETECTION METHODS IN HTS

- Spectroscopy
- Mass Spectrometry
- Chromatography
- Calorimetry
- X-ray diffraction
- Microscopy
- Radioactive methods

SPECTROSCOPY IN HTS:

- Fluorescence Spectroscopy
- Total internal reflection fluorescence (TIRF)
- Nuclear magnetic resonance (NMR)
- Absorption and luminescence
- Fourier transformed infrared(FTIR)
- Light scattering

CHROMATOGRAPHY IN HTS:

- Gas chromatography (GC)
- Thin layer chromatography
- Liquid chromatography (HPLC)

CALORIMETRY IN HTS:

- Isothermal Titration Calorimetry (ITC)
- Differential Scanning Calorimetry (DSC)

MICROSCOPY IN HTS:

- Scanning Tunnelling Microscopy
- Atomic Force Microscopy
- Confocal Microscopy

USES:

To screen Micro arrays such as:

- DNA chips
- RNA chips

METHODOLOGY

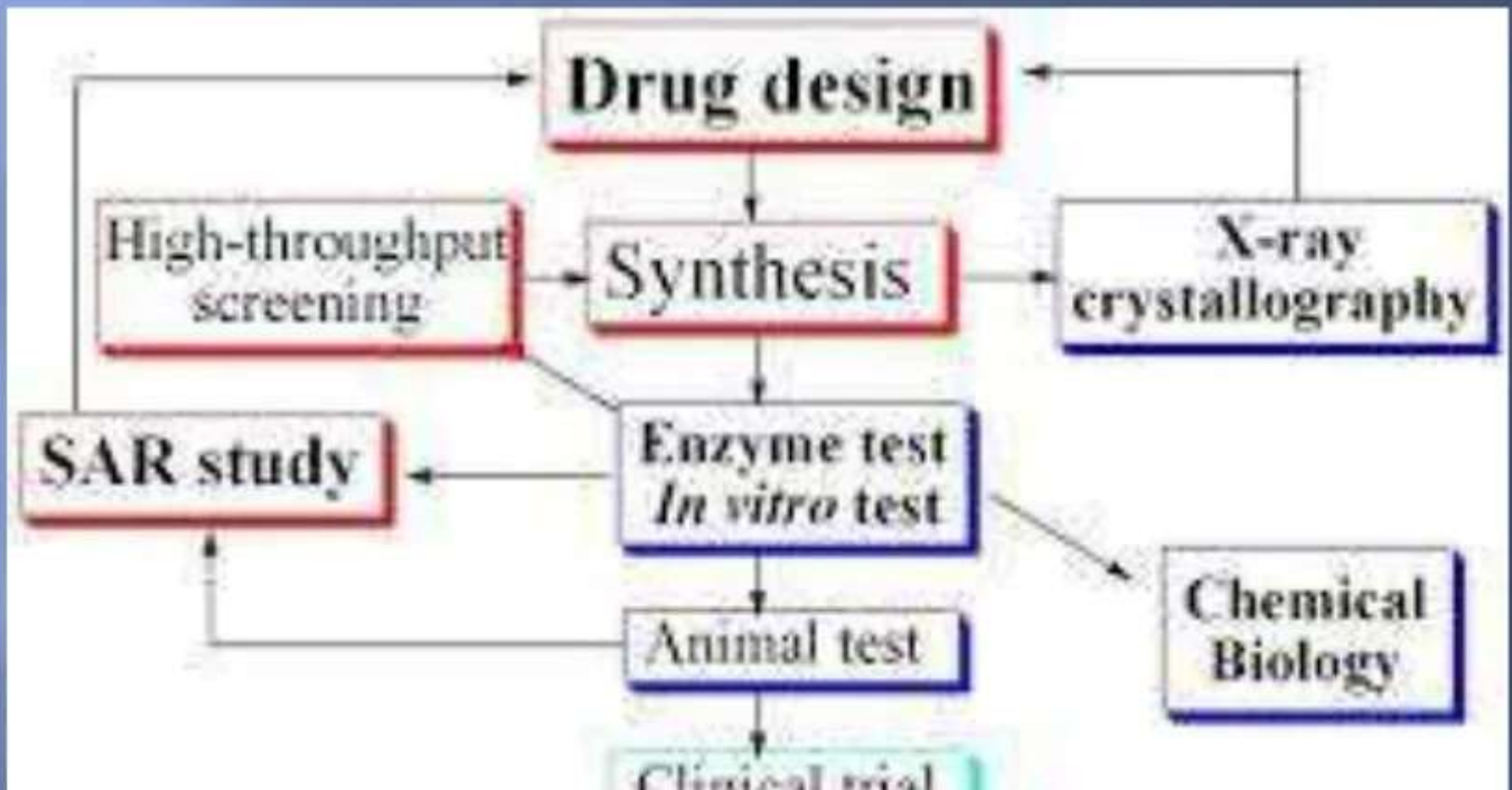
- The heart of the HTS system is a plate, or tray, which consists of tiny wells where assay reagents and samples are deposited, and their reactions monitored
- The configuration of the plate has changed from 96 wells (in a matrix of 8 rows by 12 columns) to 384, and now to a high - density 1536 - well format, which enables large - scale screening.
- Assay reagents may be coated onto the plates or deposited in liquid form together with test samples into the wells.
- Both samples and assay reagents may be incubated and

Cell - based assays have become an important test compared with other *in vitro assays*, as they can provide information about bioavailability, cytotoxicity and effects on biochemical pathway

The enzyme - based and cell - based assay systems consist of receptors or mimetics of receptors (components that mimic active parts of receptors)

Receptors- Normally the assays are linked to an indicator that shows the ligands – receptor interaction as some form of signal

The advantage of cell - based assays over biochemical assays is that cell - based assays enable the analysis of sample compound



CELL BASED ASSAYS

- ❖ Cell-based assays refer to any of a number of different experiments based on the use of live cells
- ❖ This is a general definition and can include a variety of assays that measure cell proliferation, toxicity, motility, production of a measurable product and morphology
- ❖ Cell-based assays offer a more accurate representation of the

- **FOUR KEY ELEMENTS OF CELL BASED ASSAY:**

- ✓ A cellular component e.g. a cell line or a primary cell population
- ✓ A target (substrate) molecule that records the cellular response
- ✓ An instrument to conduct and monitor the assay An informatics component to manage and analyse data from the assay
- Cell-based reporter assays are used where human receptors are transfected into null cell lines either alone, (luciferin-luciferase) or light transmission (melanophore), that can be measured independently of radioactivity within minutes.



ADVANTAGES

- ❖ **Assays do not require purification of the target protein**
- ❖ **Can immediately select against compounds / potential drugs that are generally cytotoxic, or that cannot permeate cellular membranes to reach intracellular sites**
- ❖ **Hit/lead compounds identified by cell based assays have passed important validation steps, saving time and costs in drug development**
- ❖ **Cell-based assays visualize all possible drug-target interactions e.g. activators, target interactions**
- ❖ **High sensitivity of assays & Automation**

Newer method in HTS

❖ High-Throughput, Fluorescence-Based Screening

(a) For screening, expressed proteins are labelled either as fusions with green fluorescent protein (GFP) or through translational incorporation of a fluorescent amino acid derivative, BODIPY-FL-Lysine.

(a) Using fluorescence detection, the entire procedure can be carried out in approximately 8 h.

Applications

❖ In drug discovery

❖ Systematic Study of Mitochondrial Toxicity of

CELLULAR COMPONENTS

- ❖ Different cell lines are being used in cell based assays
- ❖ Some examples are:
 - HUMAN CELL LINES
 - DU145, PC3, Lncap (Prostate cancer)
 - MCF-7, MDA-MB-438, T47D (Breast cancer)
 - THP-1 (Acute Myeloid Leukemia)

REFERENCES

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THANK YOU