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High Throughput Screening for New Drug Discovery

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ABSTRACT

The world pharmaceutical market, which was valued at $247.9 billion in 1994, is forecasted to grow to $342 billion by 1999. High throughput screening(HTS) is attracting attention as a novel methodology for new drug discovery. HTS is expected to expand the scale from one thousand to one hundred thousand times the current level by utilizing robots, laboratory information management systems(LIMS), various sources to screen natural products(plant extracts, secondary microbial metabolites), peptide combinatorial libraries and combinatorial organic synthesis(COS) for new therapeutics. Instrumentation, target selection, source material, sample preparation, primary and secondary assays, isolation, purification and structure elucidation are all important for HTS.

Key words: High throughput screening(HTS), drug discovery, laboratory information management systems(LIMS), natural products discovery, secondary microbial metabolites, combinatorial chemistry, peptide combinatorial libraries, combinatorial organic synthesis.

INTRODUCTION

The world pharmaceutical market, which was valued at $247.9 billion in 1994, is forecasted to grow to $342 billion by 1999, representing a compound annual growth rate(CAGR) of 7.1% over the 1995-1999 period from 1990-1994. There was evidence of growth in the South East Asian area(+9%) led by the booming economies of South Korea, Taiwan and Vietnam, and the Chinese market is presently showing signs of rising up, with a forecasted CAGR of 16.7% over the next five years, according to the report[1]. The world pharmaceutical market will continue to grow slowly(about +4.55% in 1995), reflecting increased government attention to escalating health care costs, enhanced by the global recession and declining tax receipts. The medium- to long-term forecast suggests that the pharmaceutical industry will see a CAGR of 7.1% as the world economy begins to improve and the benefits of global trade agreements filter through. World market growth is forecasted in the report as follows:

Forecast Growth of World Pharmaceutical Market

	1995	1996	1997	1998	1999
Total world market($bill)	259.6	273.0	296.6	318.2	342.0
Growth over previous year(%)	-	+5.1	+8.6	+7.3	+7.5

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The discovery of novel, small molecular pharmaceuticals or product candidates through screening various sources such as plant, secondary microbial metabolites, peptide combinatorial libraries and combinatorial organic synthesis (COS) expanding activity throughout the pharmaceutical industry and one that is becoming increasingly fruitful as evidenced in widespread acceptance for this virtually unlimited source of novel structures with many potential therapeutic applications.

I. *High Throughput Screening(HTS)*

Since 1986, cost-effective methodology with expertise for running natural products, plants and chemical libraries for drug discovery has been developed. In 1993, approximately more than 10,000 sample through-put could be termed as, " High Throughput Screening (HTS)", that is to say more than 40 targeted assays x 10,000 samples/project. The capability of high throughput screening in early 1995 has been reported as upgraded to 40,000-2,000,000 per assay per annum.

HTS is attracting the attention as a new methodology for new drug discovery. As screening is essential for new drug research, HTS is expected to expand the scale from one thousand to one hundred thousand times the current level by utilizing robots and laboratory information

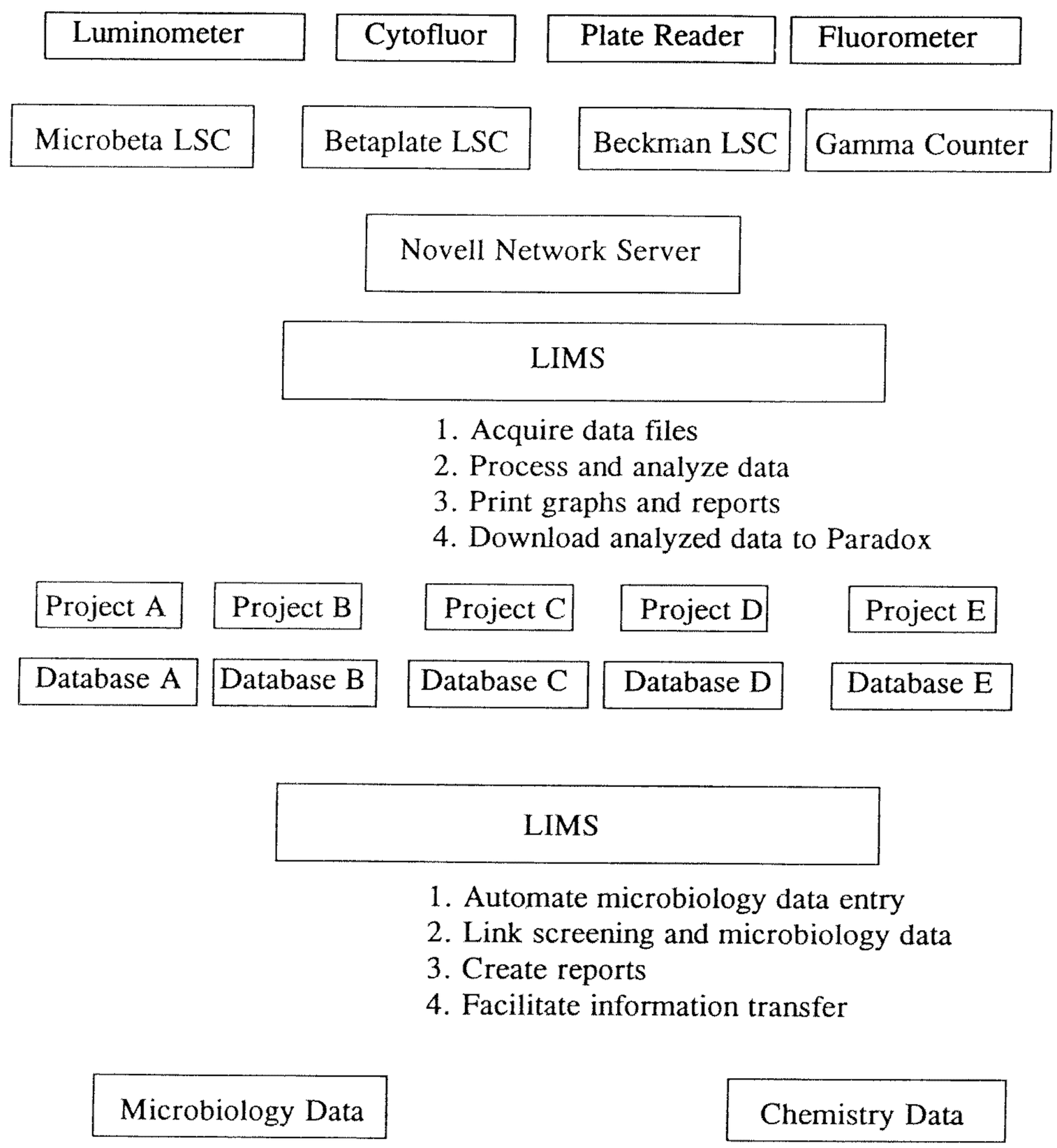

Figure 1. LIMS organization.

management systems(LIMS) to screen various sources from natural products, peptide combinatorial libraries and combinatorial organic synthesis(COS), virtually any assay system available and virtually any screening sample for new drug discovery.

Ⅱ. *Laboratory Information Management Systems(LIMS)*

Laboratory Information Management Systems is an exciting and efficient approach to laboratory processing which steps beyond basic sample tracking to increase capability and improve lab effectiveness. It is built on a solid foundation of high-performance hardware and industry-standard stand-alone software which is designed to model actual laboratory routine and implement optimum steps of automation. This maximizes the return-on-investment in automation systems. For example, in Figure 1, LIMS is tailored to include the most efficient processes whereby results can be obtained by implementation of procedures management, from sample preparation to instrument operation, and from data acquisition to the generation of reports.

Ⅲ. *Natural Products Discovery*

Naturally occurring secondary microbial metabolites and plants are well established as a major discovery source* for tomorrow's therapeutics(see Table 1). Natural products are characteristically different from those derived by chemical synthesis, and can complement current research and broaden the discovery horizons.

Screening secondary microbial metabolites from antimicrobial activity, which has existed for more than 50 years, has generated many important antibiotics. In the middle 1960's, the late H. UMEZAWA of the Institute for Microbial Chemistry in Tokyo began screening molecules from microbial sources with activities as selective enzyme inhibitors which has resulted in numerous therapeutic products[2-7]. UMEZAWA's success led to the expansion of microbial metabolite screening for non-antibiotic drug discovery. More than 15 years ago, WOODRUFF[4] reasoned that secondary microbial metabolites containing meaningful structural novelty and diversity could be accepted as a favored source for exploitation of molecules that interact with mammalian receptors, signal transduction or biosynthetic pathways which represent potentially important drug targets.

There are a large number of major pharmaceutical companies presently engaged in screen-

Natural Products with Pharmacological Activity:

Organism	Product	Activity
Aspergillus alliaceus	Asperlicin	Cholecystokinin Antagonist
Aspergillus terreus	Lovastatin	HMG-CoA Reductase inhibitor
Streptomyces avermitillis	Avermectins	Helminthic
Streptomyces tsukubaensis	FK-506	Immunosuppressant
Excellospora viridilutea	SK2315A & B	Reverse Transcriptase Inhibitors
Cladosporium cladosprioides	Calphostin C	Protein Kinase C Inhibitor
Streptomyces gabonae	MY336-a	β -Adrenergic Antagonist
Streptomyces phaeofaciens	FR-900452	Platelet Activating Factor Antagonist
Castanopermum australe	Castanospermine	α -Glucosidase I Inhibitor
Camothea acuminala	Hydroxycamptothecin	DNA Topoisomerase I Inhibitor
Streptomyces misakieusis	BE-18257B	Endothelin Receptor Antagonist
Streptomyces sp. No.7338	WS-7338	Endothelin Receptor Antagonist

ing microbial metabolites. From an economic perspective, the return on investment from this activity is widely perceived as having a high benefit to risk ratio[2]. The revenues from such products as the avermectins(discovered in a collaboration between Merck & Co. and Kitasato Institute, Tokyo), lovastatin(Merck & Co., Inc.), pravastatin(Sankyo Co., Ltd.) cyclosporin A (Sandoz, Ltd.), FK-506(Fujisawa Co., Ltd.) and numerous other candidates under development, would obviously justify the costs and efforts required to find and develop these products. Another perspective of costs and rewards is provided by the discovery of the HMG-CoA reductase inhibitor, lovastatin, which was found after only three weeks of screening soil microorganisms[3].

Despite the expanding success of the approach to drug discovery, it should be kept in mind that the vast bulk of the work is conducted in the proprietary R & D environs of pharmaceutical companies. Therefore, even although more new leads of pharmacological interest may have been discovered, for example: Ciba-Geigy, SWPRD, Zymogenetics, and Wyeth-Ayerst have published some of the research results [8-17], many of the interesting compounds may have been kept confidential for various business reasons.

Table 1. Natural product source material

(1)Plant/Marine organisms/Microorganisms (actinomycete, fungal, bacterial, marine microorganisms and etc.) or extracts from the above sources.
(2) Potential Inoculum Sources: soil, sediments, plant litter, crop residues, marine macroorganisms, dung, water, and air.
(3) Microbial Diversity:
  (A) Collection of samples from diverse geographical locations, from diverse environmental conditions, from diverse microbial habitats.
  (B) Isolation of microorganisms by pretreatment of sample, by selective pressures on growth, by colony characteristics.
  (C) Selection of microbes for screening by isolation of fresh cultures.

Ⅳ. *Applications of Combinatorial Technologies to Drug Discovery*

(Ⅰ) *Background and Peptide Combinatorial Libraries*[18].

Recent trends in the search for novel pharmacological agents have focused on the preparation of "chemical libraries" as potential sources of new leads for drug discovery. Chemical libraries are intentionally created collections of differing molecules. Combinatorial chemistry is a type of synthetic strategy which leads to large chemical libraries. Combinatorial chemistry may be defined as the systematic and repetitive, covalent connection of a set of different "building blocks" of varying structures to each other to yield a large array of diverse molecular entities. Theoretically, the number of possible different individual compounds, N, prepared by an ideal combinatorial synthesis is determined by two factors: the number of blocks available for each step "b", and the number of synthetic steps in the reaction scheme, x. If an equal number of building blocks are used in each reaction steps, then $N=b^x$. If the number of building blocks for each step varies(e.g., b, c, d in a three-step synthesis), then N=bcd. Exploitation of a basis set of (for example) 100 interchangeable building blocks permits the theoretical synthesis of 100 million tetrameric or 10 billion pentameric chemical entities.

1. *Peptide Combinatorial Libraries*

"Tens of millions of short peptides can be easily surveyed for tight binding to antibody, receptor or other binding protein using an "epitope library". The library is a vast mixture of filamentous phage clones, each displaying one peptide sequence on the viron surface. Potential application of the epitope library include investigation of the specificity of antibodies and dis-

covery of mimetic drug candidates[19]".

It is not always possible to work on an important target with structural details available such as those provided by x-ray crystallography. In contrast the traditional random screening methods, the chemical library approach is a fast way of getting to a good lead structure. In fact, the combinatorial method itself is an activity-based structural search. The advantage of using a combinatorial library is the possibility of examining a vast number of structures in a relatively short period of time.

2.*Nonpeptide Libraries*

Peptides are not ideal pharmaceuticals due to their plasma instability and transport problems. Most high affinity peptide ligands isolated from peptide library screening are not, as such, suitable drug candidates. Further rounds of molecular modeling and structure-activity relationship studies are necessary to convert the peptide lead structure into small drug molecules. The next logical step is to set-up chemical libraries of non-peptide components to screen for ligands that bind the target receptors. Through this process, the final conversion from a tight-binding peptide ligand to a potential drug candidate will become much less laborious. However, any non-peptide combinatorial library must rely on organic chemistry to a large extent, and advantage of using biological display systems for selection and amplification may not be possible[19-22].

(Ⅱ) *Combinatorial Organic Synthesis(COS)*[20]

"The notion of creating huge, searchable libraries of small organic molecules is unprescedented in medicinal chemistry, and the possibility of doing so has recently captured the imagination of the drug-discovery community. The conventional paradigm of small molecule lead development, in which a compound undergoes many rounds of individualized, hand-crafted modification and biological testing en route to drug candidacy, will likely be dramatically accelerated by the application of combinatorial chemistry technologies to mass-produce and evaluate lead analogs. Combinatorial organic synthesis(COS) presents somewhat of an intellectual inversion of the past 50 years of synthetic organic chemistry. The goal of COS are to create *population* of molecular structures—a stable population of low molecular weight entities, free of reactive and toxicity-causing functionality" [20].

A system synthesizes large numbers of chemicals rapidly and inexpensively. The chemicals are small organic compounds of diverse structure with molecular weights from 150 to 500. The chemicals are designed specifically for drug discovery screening. The key characteristics of the system are as follows:

1. *Synthesis:* using well-established chemical reactions that are thermodynamically-favored. Each chemical reaction includes two reactants: (A) a "substrate" and (B) a "reagent" which are chemically reactive. Combine one substrate with a molar excess of one reagent to synthesize the "reaction product "; although there is no further purification at this stage, yet, the material is suitable for screening. This can generate the maximum yield of product with a wide range of different substrates and reagents. Many thousands of well-established chemical reactions are applicable.

2. *Chemical/Biological Quality Control:* for quality assurance, 5% of the reaction products were analyzed by mass spectrometry to find the molecular ion of the expected product in >92% of the samples. In 70% of the samples, the expected molecular ion is the major peak in the mass spectra. Also needed is tests of the compatibility of the reaction products with several different high-throughput screening assays including different receptor-ligand binding assays, different enzyme inhibition assays, and different whole cell assays. These assays employ either fluorometric or radiometric end points.

3. *Combinatorial Organic Synthesis:* for example, if we select 100 substrate and 10 reagents, we can synthesize 1,000 separate reaction prod-

ucts. Likewise, based on only 10 different reactions and chemicals available from a chemical supplier these may generate over 2,000,000 reaction products. Since the principle of combinatorial chemistry is the assembly of a large collection of structurally diverse monomers, a highly efficient coupling method is a critical component in the design of such non-peptide libraries.

4. *Chemical Novelty:* many of the reaction products are not commercially available and therefore cannot be purchased. They may be known in the scientific or patent literature, but they are unobtainable. Some of the reaction products may be novel and patentable as composition of matter.

5. *Custom Libraries for Lead:* often after lead is discovered from screening, scientist can broadly explore quickly and inexpensively to generate several hundred analogs and derivatives to "explode" ———the lead[23].

Ⅴ. *Important Points of High Throughput Screening*

To assure a successful High throughput screening, there are several important points needed to be noticed, such as developing efficient support instrumentation, good research planning and strategy in target selection, study implementation via profound disciplinary expertise:

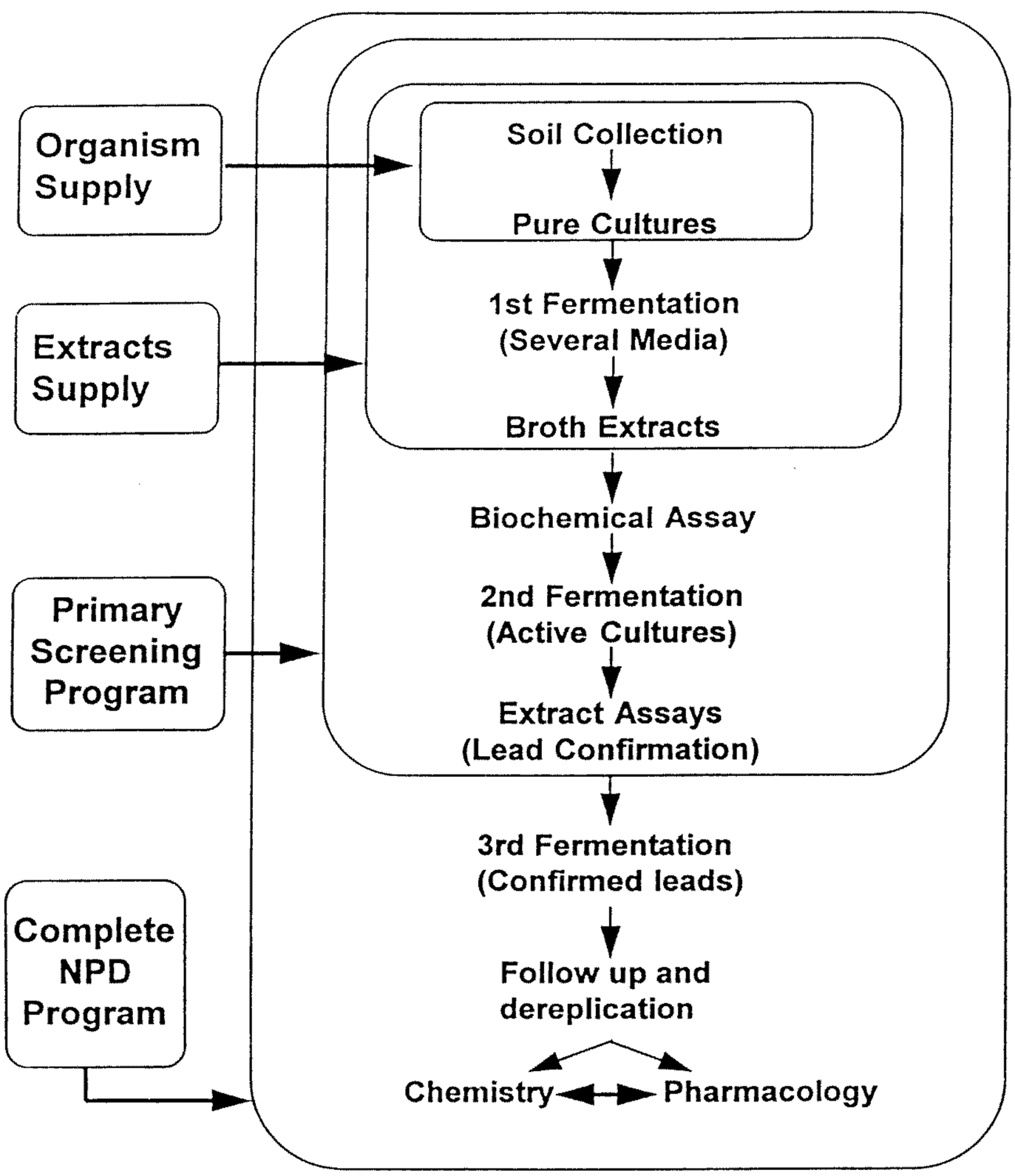

Figure 2. Flow chart for natural products discovery.

(I) *Instruments for High Throughput Screening*

1. Robots
   Biomek 1000 Pipetting Station with Sidesholder(2 sets)
   TomTec 96-Well Pipetting Station
   Packard Multi-Probe 200 Pipetting Station
   Hamilton SPE Station
2. Radioactive Counters
   LKB 1205 Betaplate(2 sets), LKB 1405 MicroBeta, LKB 1207 GammmaMaster, and Beckman 1800 LSC
3. Spectrophotometric Counters
   LabSystems Luminoskan, Millipore CytoFluor 2300, Perkin-Elmer LS-50B, and Bio-Tek Absorbance Reader.
4. Data Acquisition
   Novell Network, 25 computer Workstations, Custom Data Acquisition, Analysis Software

(Ⅱ) *Critical Steps of a Screening Program*

1. Target selection/Assay development
2. Selection of source material
3. Sample preparation
4. Primary assay
5. Secondary assays
6. Isolation and purification
7. Structure elucidation

(Ⅲ) *Targets for Novel Natural Products Therapeutics*

Receptors, enzymes, transcription assays, cell recognition molecules, ion channels, and signal transduction molecules as targets for HTS. Using radiometric analysis for receptor binding, calorimetric or fluorescence analysis for ELISA substrate/product, and reporter genes for transcription assays.

(Ⅳ) *Be Aware of False Hits*

Which may be caused by colored extracts, detergents, denaturing agents, cytoxins, and chelators.

REFERENCES

1. World Market to 1999. Scrip (No. 2026) May 19th, 1995, p.19.
2. Yarbrough, G.G., Taylor, D.P., Rowlands, R.T., Crawford, M.S., and Lasure, L.L. 1993. Screening Microbial Metabolites for New Drugs-Theoretical and Practical Issues. J. of Antibiotics. 46:535-544.
3. Vagelos, P.R. 1991. Are Prescription Drug Prices High? Science 252:1080-1084.
4. Woodruff, H.B. 1980. Natural Products from Microorganisms. Science 208: 1225-1229.
5. Omura, S. 1986. Philosophy of New Drug Discovery. Microbiol. Rev. 50: 259-279.
6. Omura, S. 1992. Trends in the Search for Bioactive Microbial Metabolites. J. Indust. Microbiol. 10: 135-156.
7. Fenical, W. 1993. Chemical Studies of Marine Bacteria: Developing a New Resource. Chem. Rev. 93: 1673-1683.
8. Roggo, B.E., Peterson, F., Delmendo, R., *Jenny, H.-B., Peter, H.H.* 1994. 3-Alkanoyl-5-Hydroxymethyl Teronic Acid Homologues: New Inhibitors of HIV-1 Protease. I. Fermentation, Isolation and Biological Activity. J. of Antibiotics. 47:136-142.
9. Roggo, B.E., Hug, P., Moss, S., Raschdorf, F., Peter, H.H. 1994. 3-Alkanoyl-5-Hydroxymethyl Teronic Acid Homologues: New Inhibitors of HIV-1 Protease. II. Structure Determination. J. of Antibiotics. 47:143-147.
10. Wong, S.-M, Kullnig, R., Dedinas, J., Appel, K., Kidd, G., Gillum, A., Cooper, R., Moore, R., 1993. Anthrotainin, an Inhibitor of Substance P Binding Produced by *Gliocladium carenulatum*: J. of Antibiotics. 46:214-221.
11. Wong, S.-M, Musza, L., Kidd, G., Kullnig, R., Gillum, A., Cooper, R., 1993. Anthrotainin, an Inhibitor of Substance P Binding Produced by the Fungus *Neosartorya fischeri*. Taxonomy, Fermentation, Structures, and Biological Properties: J. of Antibiotics. 46:545-553.
12. Barrow, C.J., Cai, P., Snyder, D., Sedlock, D., Sun, H.H., Cooper, R., 1993. WIN 64821, a New Competitive Antagonist to Substance P, Isolated from an *Aspergillus* Species: Structure Determination and Solution

Conformation. J. Org. Chem. 58:6016-6021.
13. Sedlock, D., Barrow, J., Brownwell, J., Hong, A., Gillum, A., Houck, D. 1994. WIN 64821, A Novel Neurokinin Antagonist Produced by an *Aspergillus sp.*, I. Fermentation and Isolation: J. of Antibiotics. 47: 391-398.
14. Oleynek, J., Sedlock, D., Barrow, K., Appell, K., Casino, F., Haycock, D., Ward, S., Kaplita, P., Gillum, A., 1994 . WIN 64821, A Novel Neurokinin Antagonist Produced by an *Aspergillus sp.*, II. Biological Activity: J. of Antibiotics. 47: 399-410.
15. Popp, J., Musza, L., Barrow, C., Rudewicz, P., Houck, D. 1994. WIN 64821. A Novel Neurokinin Antagonist Produced by an *Aspergillus sp.*, III. Biosynthetic Analogs: J. of Antibiotics. 47:411-419.
16. West, R., Smith, R., McKernan, P., Chen, L.H.J., Rosenberg, G., Biggs, S., Delmendo, R., Pedigo, L., Piggot, J. 1993. Isolation and Identification of a Glucagon Antagonist from an Unidentified Fungus, *Penicillium sp.*: Poster Presented at the Third International Conference on Biotechnology of Microbial Products, SIM.
17. Henrickson, D.H., Alvi, K.A., Peterson, J.R., Hosken, M.I., Baker, D., Cochran, D., Hofman, B., Leung, K.H., 1994: Identification of an Active Metabolites from *Streptomyces violaceusniger* in a Kainate Receptor Binding Assay. Poster Accepted for Presentation at the SIM Annual Meeting.
18. Gallop, M. A. 1994. Application of Combinatorial Technologies to Drug Discovery. 1. Background and Peptide Combinatorial Libraries. J. Med. Chem. 37: 1233-1251.
19. Scott, J. K. and Smith, G.P. 1990. Search for Peptide Ligands with an Epitope Library. Science. 249: 386-387.
20. Gordon, E. M. 1994. Application of Combinatorial Technologies to Drug Discovery. 2. Combinatorial Organic Synthesis, Library Screening Strategies, and Future Directions. J. Med. Chem. 37: 1385-1401.
21. Lam, K. S. 1991 A New Type of Synthetic Peptide Library for Identifying Ligand-Binding Activity. Nature 354: 82-84.
22. Houghten, R.A. 1991. Generation and Use of Synthetic Peptide Combinatorial Libraries for Basic Research and Drug Discovery. Nature 354: 84-86.
23. Peterson, J. and Paslay, J. 1995. Synthetic Chemical Libraries for Screening. Presentation at the High Throughput Screening for Drug Discovery Conference, San Diego.

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大量快速篩選與新藥開發

林稟彬

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摘　要

全球藥品市場於一九九四年市值美金二千四百七十九億元，到一九九九年市值預測為美金三千四百二十億元。在新藥開發研究方法上，大量快速篩選(High throughput screening, HTS)廣受注意。利用自動化機器、實驗室資訊管理系統，以及各種資源來大量快速篩選各種天然物（包括植物萃取、微生物代謝物）、庫存化學品、混式結合合成化學產品來尋找新藥。儀器設備、療效篩選目標之選擇、物料來源、樣品備製、初篩、複篩、有效物之分離純化、結構鑑定，皆為實施HTS之重要步驟。