MEETING THE CRITICAL REQUIREMENTS OF AUTOMATED DNA SAMPLE PREPARATION FOR BIOREPOSITORIES

INTRODUCTION

The biorepository concept is experiencing rapid growth with at least 179 biorepositories already established in the United States, according to a Piribo market research study. The current Biorepositories model entails collecting tissue samples, DNA samples and often serum or plasma samples from groups of participants targeted by population or disease. These samples along with associated demographic and clinical information are banked for future use by researchers investigating relationships between genotypes and disease. An important trend is that many medical centers and research institutions are shifting to a prospective approach of sample acquisition and changing their focus from collecting DNA samples to meet the needs of single investigator to collecting and banking samples from a wide range of patients to facilitate the work of multiple investigators. This approach requires enormous increases in the number of samples that are collected and as a result the vast majority of biorepositories have either adopted or are considering the adoption of automated sample preparation methods. The automated sample preparation equipment decision needs to be made carefully because the high volumes of samples collected and longer storage times of the sample components required in the new generation of biorepositories places a critical level of importance upon sample preparation throughput, reliability and purity. This article will explain in detail the key requirements of automated DNA sample prep for biorepositories and how they can be achieved.

SAMPLE THROUGHPUT

The numbers of biospecimens involved in today's biorepositories is exploding. For example, the Mayo Clinic has over 1.2 million samples sitting in 50 upright freezers and 25,000 new samples are being added every month. The cost and time involved in preparing samples of this magnitude for storage using traditional manual methods are almost incalculable. Automated sample preparation methods are clearly needed but should be carefully considered to keep sample preparation costs low. The extraction chemistries are the key

controlling factor in the total potential throughput of instruments which typically range from 80 to 100 samples per day. However, the amount of manpower required to operate these instruments varies considerably and it should be considered during the instrument selection process because it has a major impact on the labor costs involved in sample preparation.

The AutoGen Flex STAR can be set up in 30 minutes and will then process 40 samples without further intervention. Two batches can be completed in one work day providing a total throughput of 80 samples per day. The primary competitive instrument also requires 30 minutes of setup time but will run only 16 samples per batch. The batches take less time to run so throughput of the competitive instrument is equivalent. Feedback from users of the competitive instrument indicates that it often requires frequent attention from operators even when running in unattended mode. Thus, even though the competitive instrument will produce approximately the same number of samples per day, labor costs are considerably higher.

INSTRUMENT RELIABILITY AND SUPPORT

Equally important in considering the throughput of a potential automated sample preparation system is the availability of the instrument. Automated DNA sample preparation systems are complex instruments so their reliability and their supplier's support capabilities are critical to a successful biorepositories operation. Potential throughput rating means nothing if the instrument is sitting idle with the operator trying to figure out the error codes or waiting for the manufacturer to send a service engineer. Because reliability is so important, before purchasing any instrument, it is important to speak to current users to find out how frequently they have problems with the instrument, how often it is out of service, and how quick and how effective is the support that is provided?

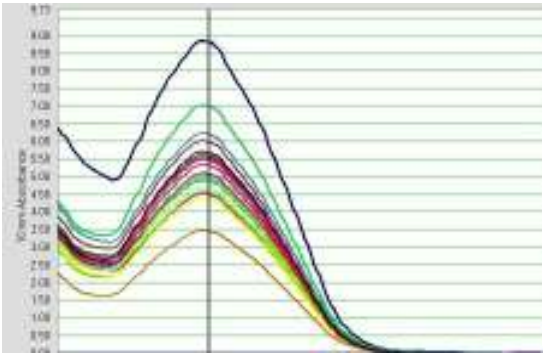
Biorepository operators that have used both the AutoGen Flex STAR and its main competitor say that the primary advantage of the Flex STAR is its higher reliability and the superior support provided by AutoGen which translates into higher throughput and lower sample costs. The Flex STAR has a simple and reliable design that is intended to provide many years of continuous operation with a relatively small amount of maintenance. Flex STAR instruments require an average of only one service call per year while users of the competitive instrument state that it typically requires 8 to 10 service calls per year. AutoGen is a small company dedicated to sample prep whose success or failure depends upon the happiness of the users of the Flex STAR and a few other products. Customers are sometimes surprised when the Chief Executive Officer of AutoGen calls them to see whether they were satisfied with a service call. On other hand, the competitive product represents only a small percentage of the revenues of a much larger company. It's also worth nothing

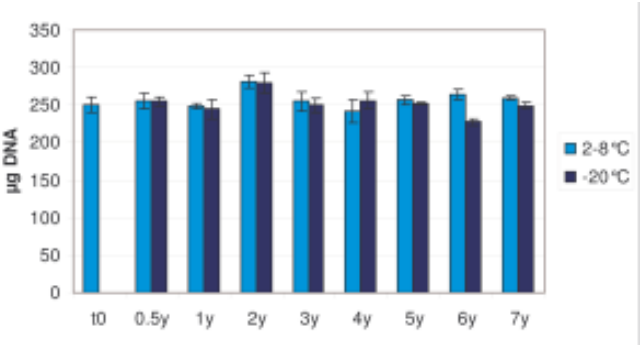
that AutoGen is an American company which in some cases makes it easier to obtain government funding of an instrument purchase.

DNA PURITY AND LONG TERM STORAGE

Purity of banked DNA sample has become more important than ever. Given the biorepositories' prospective approach to acquiring samples, the extracted DNA must remain intact and usable during long term storage. With this in mind it becomes important to remove RNA and especially proteins, nucleases and other inhibitors during the extraction process because these contaminants could lead to DNA degradation over the long periods of time the material may be stored in the biorepository. Furthermore protein contamination is known to be a potential inhibitor in PCR amplification and could lead to problems with downstream studies. Therefore, the degree of purity of the DNA produced is an important factor to consider when evaluating alternative automated sample preparation systems.

The AutoGen Flex STAR provides a pure DNA sample using proven Flexigene chemistry that employs an initial process that pellets the white cell nuclei and mitochondria while simultaneously lysing the red cells. During the next step it denatures and eliminates the proteins and cellular material utilizing a protease enzyme. On the other hand, the competitive instrument uses a process that lyses the red cells and then applies a precipating agent to the resulting white cell pellet to salt out or precipitate the proteins from the nucleic acids in a solution. Since this method does not use a protease enzyme to digest the precipitated proteins and contaminants there remains the possibility of carryover of these contaminants into the DNA solution. In addition if an RNA free sample is required an additional RNA digestion step must be performed. The amount of DNA captured by each method is similar. However, the competitor claims a higher DNA yield but is in reality measuring total nucleic acids including the RNA left over in the sample. AutoGen demonstrates the purity of its DNA samples by analyzing them with a spectrophotometer at 260/280 nm. Samples produced on the Flex STAR consistently deliver a reading between 1.7 and 1.9 which indicates pure DNA, free of contaminating proteins and RNA.





Yield: 100 - 210 µg/5 ml

Av. A_{260}/A_{280}: 1.8 – 1.9

Stability for 7 years at 2-8°C and 20°C*

INITIAL COST AND OPERATING COST

As with any other major purchase, cost is an important consideration in the instrument selection process. Large biorepositories typically utilize many automated sample preparation instruments. The AutoGen Flex STAR has a list price under $150,000 while the competitive instrument lists out at $250,000. The list price of a service contract for the Flex STAR is $15,340 per year compared to over $25,000 for the competitive instrument. Operating costs per ml of sample are $1.30 for the Flex STAR vs. $1.60 for the competitive instrument. Based on a workload of 100 samples per week the operating costs for the Flex STAR will be $7800 less than that of the competitive instrument. The AutoGen Flex STAR provides additional savings by conserving laboratory space. It occupies a footprint of only 36 inches wide by 29 inches deep by 59 inches high compared to 75 inches wide by 32 inches deep by 68 inches high for the competitive instrument.

Comparison of AGF STAR vs Competitor

	AGF STAR	**Competitor**
Size of instrument	36”(w) x 29” (D) x 59” (H)	75” (W) x 32” (D) x 68” (H)
Weight of Instrument	250 kg	700 kg
Ease of use	Very simple to set up, start up and operate	Very complex instrument requiring dedicated computer
Maximum samples per batch	40	16
Contaminants in prep	None only pure DNA	RNA, protein
Service calls per year	1	12

CASE STUDIES

MAYO CLINIC

“The Mayo Clinic biorepository has evolved over the past 15 years,” said Jason Carnahan, Supervisor of the Biospecimen Accessioning and Processing Shared Resource. “In the past few years the concept has gained a lot of traction and volumes have exploded. Originally our focus was single investigator based studies where the investigator set up a protocol and study coordinators mailed out kits to collect samples from the prescribed population. More recently we have moved to a model where we collect samples from a wide variety of patients. Investigators can utilize our biorepository to begin work immediately on a study.” This resulted in a high demand to produce DNA from storage from an ever increasing influx of samples.

Carnahan said that the organization had concerns with their original automated sample preparation instrument including reliability and turnaround time for repairs. “When we heard that one of our research operations had purchased the AutoGen Flex STAR we flew down to see it,” Carnahan said. “We were very impressed with the instrument. Our sister organization praised its reliability and AutoGen demonstrated their willingness to work with us to meet our needs. We like the fact that the software is open so that we can change parameters and protocols. We are saving considerable amounts of time because we can set up the Flex STAR to process 40 samples in the same amount of time that it previously took us to set up the old instrument for 16 samples. These savings are important because labor is by far the largest expense in running a biorepository.”

“But the primary reason that we have been so happy with the Flex STAR is its reliability. We often felt that our previous instrument required babysitting for sample loading and error correction. The large amount of attention that was required made us question our investment in automation. On the other hand, we can set up the Flex STAR and walk away or even go home with complete confidence that it will run through the protocol and provide DNA in solution ready for banking. Furthermore, I have been working in bioscience for 15 years and have never seen a company respond as quickly and completely as AutoGen. It’s a bonus that the Flex STAR, including its service contract, is considerably less expensive. We ran the two different instruments side by side for a trial period and then made the decision to operate exclusively with the Flex STAR. Today we have three Flex STARs in operation and one more on order.

MT. SINAI MEDICAL CENTER

Mt. Sinai Medical Center started its biorepository in 2006. “When we started operation our choices for DNA extraction from large volumes of whole blood were limited because only one automated DNA sample preparation instrument was available,” said Greg Khitrov, Assistant Professor of Medicine and Director of the Institute for Personalized Medicine Core Laboratories at the Mt Sinai Medical Center. “We purchased that instrument but we had several problems. The instrument failed frequently and we had problems with protein contamination that made it difficult to successfully perform PCR with the DNA sample.”

“When the AutoGen Flex STAR came onto the market we tried it out and decided to purchase the instrument,” Khitrov added. “Since then we have literally had only a handful of failures and most were the result of our operators becoming familiar with the instrument. Support has also been very good. The DNA produced by the new instrument has been very clean. Based on our success with the Flex STAR we have retired and are in the process of selling our original instrument.”

Mt. Sinai typically draws 8 to 10 ml of blood and then pulls out 2 milliliters of plasma leaving 6 to 8 ml of white blood cells and serum. They run 3 ml samples in the Flex STAR so in the rare event of a problem they have enough blood left to run another sample. Mt. Sinai is at present running a 1200 patient GWAS study that involves looking at samples from participants currently enrolled in its biorepository. The samples are used on Affymetrix GeneChip 6.0 genotyping chips.

CONCLUSION

An estimated $1 billion has been invested in the biorepositories industry in the last few years. Studies enabled by biorepositories are expected to provide improvements in diagnosis and treatment of cancer as well as other common diseases. The advancement of the biorepositories industry depends upon automated DNA extraction systems that are able to deliver high levels of pure DNA to ensure sample integrity while providing low setup time and high reliability to keep costs at a reasonable level. The AutoGen Flex STAR meets this challenge by, providing pure DNA samples that are free of contamination from proteins and nucleases that can potentially inhibit PCR amplification and cause DNA degradation over time. The Flex STAR also produces much larger batches with the same amount of setup which reduces labor expenses. Finally, users of the Flex STAR have commented that the instrument is more reliable than the main alternative and that AutoGen provides superior support. Overall the Flex STAR system provides higher purity DNA, at 25% lower operating costs with 10 X more uptime than the competitive system. This is the formula for successful sample preparation in today's biorepository.