New approaches to sample identification tracking and technologies for maintaining the quality of stored samples

By Dr John Comley

Safeguarding the quality of stored samples (both compounds and biospecimens) must be the driving ethos in compound management stores and biorepositories. Yet there are few innovations in available sample storage technologies that can help them easily identify poor sample quality or monitor sample degradation and so minimise erroneous downstream analytical results. Recent advances in sample ID and tracking undoubtedly give much greater confidence upholding an unbroken chain of custody. New storage tube configurations will contribute to enhanced screening efficiencies and greater reliability and advanced data management systems can improve overall sample intelligence. However, none of these technologies give reassurance that the stored samples are actually fit for their intended purpose. A MEMS-based sensor chip incorporated into storage tubes or vials that can monitor temperature and ID tracking over the sample lifecycle has the potential to stratify samples into fit-for-purpose categories based on their previous temperature exposure history. However, tracking sample temperature by itself will not improve on the quality of samples that were of intrinsically poor quality at the outset, and will only enhance the integrity among stored samples if there is willingness to act upon the results of 'outside set range' temperature sensing. What the industry is lacking are simple and fast QC/QA verification tests that can confirm a sample is what is supposed be or if it has suffered degradation. Until such tests become routine, storage facilities and end-users of stored samples need to do all they can to try to avoid processing variables which have the potential to impact sample integrity.

Preserving the quality of stored samples is of fundamental importance in compound management stores and biorepositories. In recent years the significance of maintaining sample integrity has become a major consideration, not least because it has the potential to impact most downstream applications. Variability, a precursor to poor sample integrity, can be introduced at any stage of the storage process, whether it be during sample collection, shipping, extraction, dissolution, aliquoting, cryopreservation or the long-term storage itself. Therefore, the goal must be to preserve integrity at every step of the workflow and ensure samples make it from the patient to repository to analysis, or from compound synthesis to liquid solution to assay, in the most consistent way that is fully documented.

Other contributing factors to poor sample integrity include degradation, sample instability and hydration, often arising from freeze-thaw cycling. These have largely been addressed by freezing multiple single-use aliquots in mini-tubes, although the ability to extract an aliquot from a frozen parent sample or tissue without freeze-thawing is advantageous in some circumstances.

Ensuring reliable sample information, tracking and security is particularly challenging with storage at low temperatures (-80°C and below). Gone are the days where human readable written labels and data recorded in an Excel spreadsheet are sufficient, except for the most basic inventory requirements with a very limited number of stored samples. Linear and 2D barcodes have undoubtedly improved identification (ID) and storage tracking although they are subject to 'line of sight' issues caused by frost, ice, condensation or fog build-up on the scanning platform, the permanency of some attached codes or labels cannot be guaranteed and are subject to the occasional barcode flaw. These issues have led to a new generation of smarter tags (some based on radio frequency identification (RFID), electronic micro transponders, and micro-electro-mechanical system (MEMS) technologies) which provide a robust indelible ID code for unbroken chain of custody, that are resistant to various methods of sterilisation (eg gamma radiation and autoclaving) and capable of withstanding and operating at ultra-low cryogenic (-196°C) temperatures.

Tags can be attached to an individual sample or to the sample transport container, and some tags (eg Bluechiip®) even enable the temperature to be measured, time-stamped and recorded with the sample ID at the point of storage. This allows the temperature and ID history to be tracked over the sample

Figure 1: Main applications or intended uses of stored samples of respondents surveyed

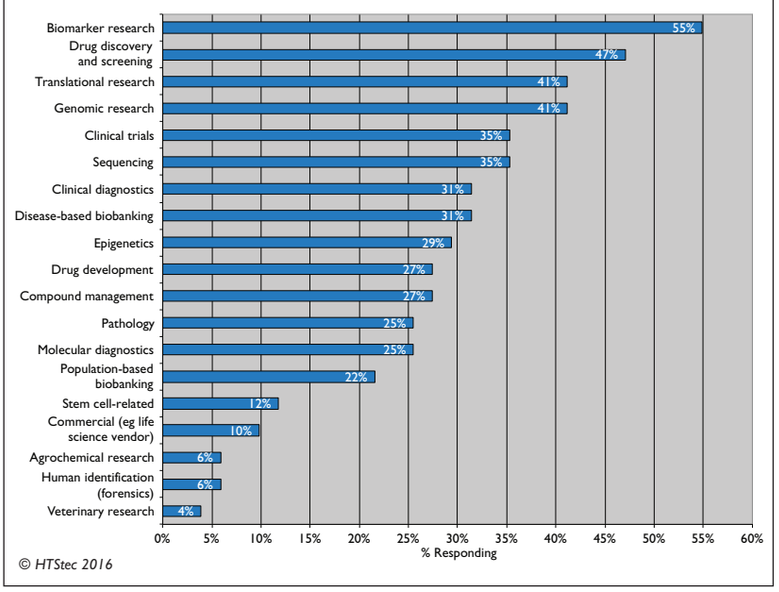


Figure 2: How respondents track their samples today to ensure reliable information, location and quality

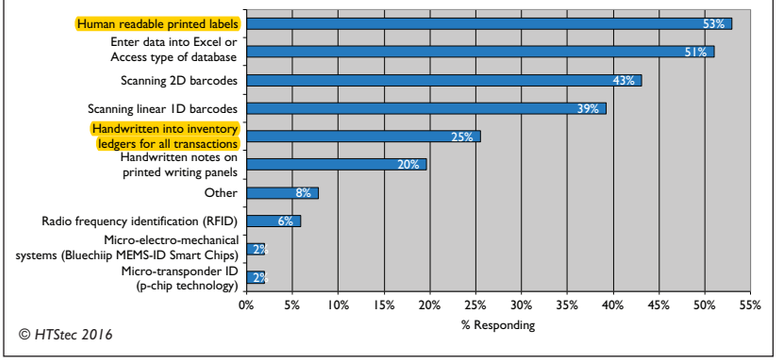


Figure 3: How respondents verify the quality of their stored samples today

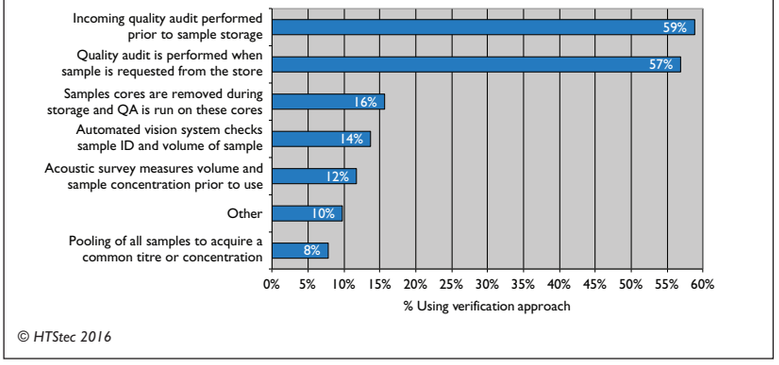


Figure 4: Level of satisfaction with quality and chain of custody tracking technologies

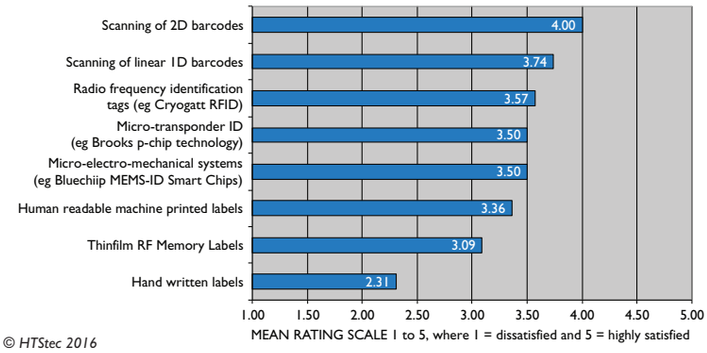


Figure 5: Extent to which obstacles limit respondents' use of stored samples

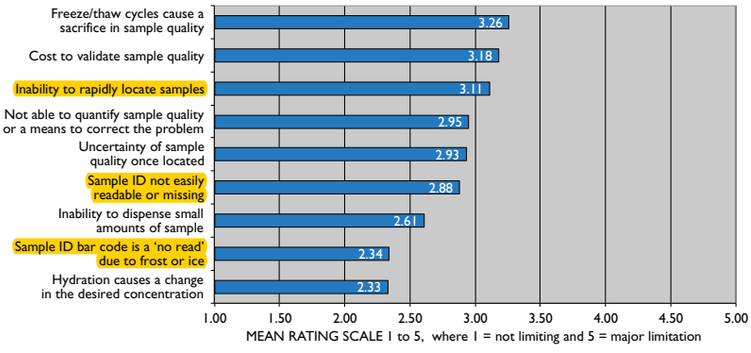
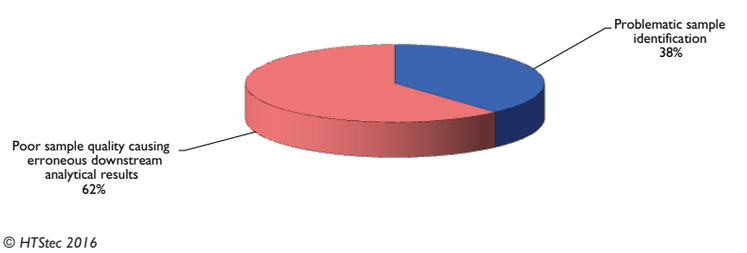


Figure 6: Problem perceived as most damaging to respondents' expected use of stored samples



lifecycle and 'outside set range' flags assigned at the sample or transport container level to alert the end-user of potential storage temperature fluctuations that could impact quality. Ultimately such an approach might facilitate the stratification of samples into fit-for-purpose categories.

In October 2016, HTStec undertook a market survey on the emerging requirements for ID tracking and maintaining the quality of stored samples[1]. The objectives of the survey were to understand current methods used for ID tracking of stored samples, the importance of sample quality and of approaches used to maintain quality, and to establish potential interest in new sample ID and quality tracking technologies. In this article highlights from the market survey are reported and the findings are discussed together with vendor updates on the latest on sample tracking technology and new tools for maintaining sample quality.

Main applications of stored samples

The main application or intended use of stored samples by most (55%) respondents surveyed was biomarker research. This was followed by 47% drug discovery and screening; 41% translational research; and 41% genomic research. Least used applications include agrochemical research, human identification (forensics) and veterinary research (**Figure 1**).

Current approach to sample tracking ID and quality verification

How respondents track their samples today to ensure reliable information, location and quality is presented in **Figure 2**. This showed that survey respondents most used human readable printed labels to track their samples today (53% using). This was closely followed by enter data into Excel or Access type of database (51% using); scanning 2D barcodes (43% using); and then scanning linear 1D barcodes (39%). Current implementation of newer tracking technologies (eg RFID, p-chip and Bluechiip) among survey respondents was minimal (**Figure 2**).

With respect to verifying the quality of their stored samples, most survey respondents either perform an incoming quality audit prior to sample storage or a quality audit is performed when a sample is requested from the store. All other verification approaches were only used by a minority of respondents (**Figure 3**).

Survey respondents' level of satisfaction with quality and chain of custody tracking technologies was rated highest (most satisfied) with scanning of 2D barcodes, and then scanning of linear 1D

barcodes. Rated lowest (most dissatisfied) with was handwritten labels. The rating of some of the more recent tracking technologies (eg RFID, p-chip and Bluechiip) was moderately satisfied, but the number of respondents familiar enough or using these technologies to respond was limited (**Figure 4**).

Main causes of poor sample quality

Survey respondents rated freeze/thaw cycles causing a sacrifice in sample quality as the main obstacle that most limits the use of stored samples. This was followed by cost to validate sample quality; inability to rapidly locate samples; and then not able to quantify sample quality or a means to correct the problem. Rated least limiting were sample ID barcode is a 'no read' due to frost or ice and hydration causes a change in the desired concentration (**Figure 5**).

The majority (63%) of survey respondents reported that poor sample quality causing erroneous downstream analytical results was the most damaging problem to their expected use of stored samples. The remainder (38%) of the respondents reported that problematic sample identification was the most damaging problem (**Figure 6**).

The number of times samples are exposed to a significant temperature change per day is presented in **Figure 7**. The median number of significant temperature changes survey respondents reported their samples were exposed to was one change per day. This daily change in temperature undoubtedly has an adverse effect on sample quality.

Most (43%) survey respondents current use protocol adopted for stored samples in their facilities was single-use aliquots in vials, tubes or straws, etc and any remaining samples are discarded. This was followed by samples are checked in and out of our stores multiple times and we may see a loss in sample quality (30%); samples can be checked in and out of stores multiple times and we are aware of no loss in sample quality (20%); and then samples are checked in and out of our stores multiple times and we know sample quality is compromised (7%) (**Figure 8**).

Whether stored samples were subject to standardised standard operating procedures (SOPs) prior to storage is reported in **Figure 9**. This showed that most survey respondents prepared their samples either off-site or on-site according to strict SOPs to minimise pre-analytical variables (27% using either). This was followed by samples prepared on-site under variable conditions as they appear to be unavoidable (25% using) and then samples prepared off-site under variable conditions beyond our control (17% using).

Figure 7: Number of times samples are exposed to a significant temperature change per day

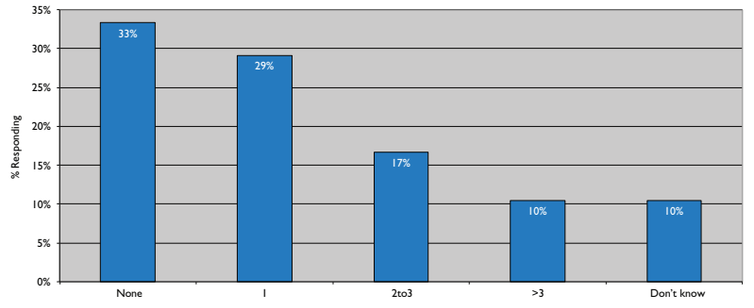


Figure 8: Current use protocol for stored samples adopted in respondent's facility

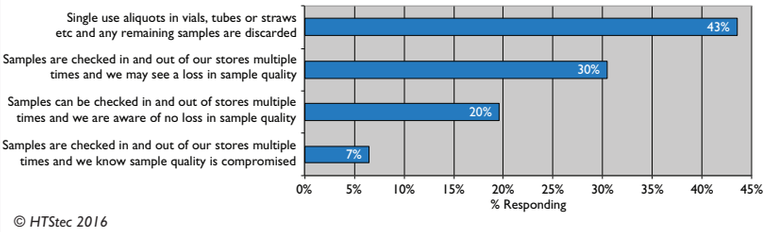


Figure 9: Whether stored samples were subject to standardised SOPs prior to storage

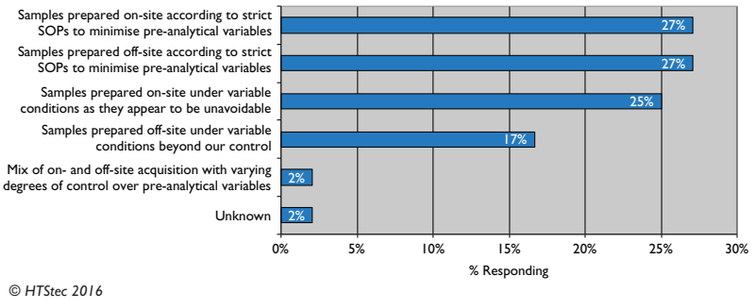


Figure 10: Aspects of the sample deposition and storage process respondents felt most critical to their group's success

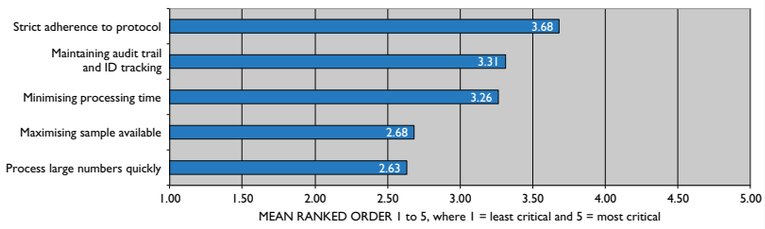


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Figure 11: Valued placed on a continuous audit trail of sample ID and temperature excursions

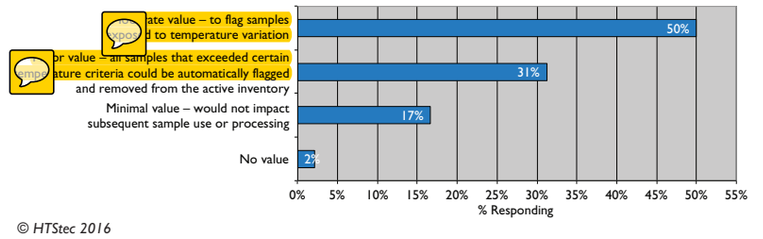

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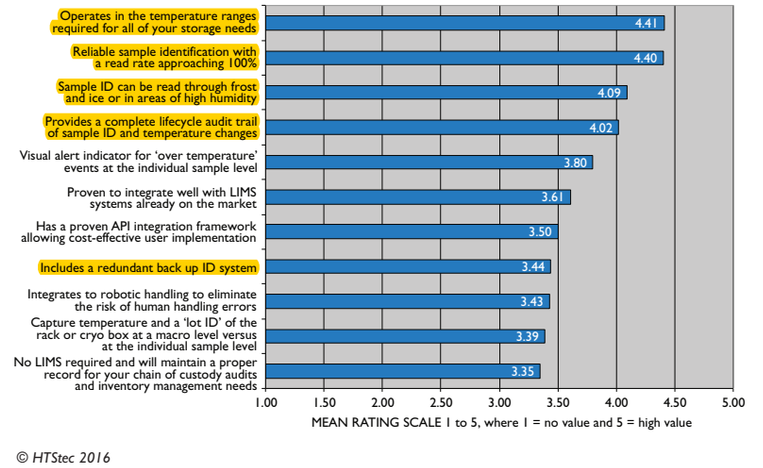
Figure 12: Most valuable features wanted in any new sample ID and quality tracking technology



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Improving stored sample quality

Survey respondents ranked strict adherence to protocol as the aspect of the sample deposition and sample storage process they felt most critical to their group's success. This was followed by maintaining audit trail and ID tracking; and then minimising processing time. Ranked the least critical was process large numbers quickly (**Figure 10**).

Most (50%) of survey respondents placed moderate value (ie to flag samples exposed to temperature variation) on a continuous audit trail of sample ID and the temperature excursions a sample has been exposed to during its lifecycle. This was followed by major value (ie all samples that exceeded certain temperature criteria could be automatically flagged and removed from the active inventory) (31% using); minimal value (ie would not impact subsequent sample use or processing) (17% using); and then no value (2% using) (**Figure 11**).

The most valuable features wanted in any new sample ID & quality tracking technology is presented in **Figure 12**. This showed that survey respondents rated operates in the temperature ranges required for all of your storage needs as the most valuable feature wanted in any new technology. This was closely followed by reliable sample identification with a read rate approaching 100%; and then sample ID can be read through frost and ice or in areas of high humidity; and provides a complete lifecycle audit trail of sample ID and temperature changes. Rated least value was no LIMS required and will maintain a proper record for your chain of custody audits and inventory management needs.

Latest vendor developments

The following vendor snapshots describe some of the latest developments in sample identification tracking and technologies for maintaining the quality of stored samples.

Bluechiip (www.bluechiip.com) provides wireless ID tracking and temperature sensing technology that survives and operates in extremely harsh environments such as cryogenic temperatures and ionising radiation. The offering comprises hardware, software and engineering support targeting both end-users and OEM partnerships. Bluechiip and its licensees are the only vendors that offer a MEMS-based technology to track samples. The hardware consists of the actual ID chip and the reader. The chip can have different packaging configurations to suit a wide range of applications and encapsulation processes. As an example, the Bluechiip tag can be co-injected in vials, mechanically attached with

glue or just loosely enclosed in a cavity. The antennas can also have a multitude of shapes depending on size constraints. On the other hand, the software offering is a user-friendly database that can work as a Sample Data Management web application for end-users or can also be accessed through Web API for a full LIMS integration into partner products. Bluechiip technical support offers engineering and aftersales support for all customers (**Figure 13**).

In today's biomedical research environment, biobanks are a repository for the physical sample, but increasingly also for the exponential amount of data tied to each sample. Further, the type and amount of data is multiplying and the information desired by researchers is becoming more broad and complex. As a result, many research organisations are seeking technologies and strategic processes to improve overall sample intelligence and increase the global visibility and quality of the samples within their distributed biobanks. In response, **Brooks Life Sciences** (www.brooks.com/life-science) recently launched its BioStudies® Biobank Data Management System. Designed specifically to integrate sample inventory and other data related to samples from disparate data sources, BioStudies connects sample inventory, consent, phenotypic and clinical data, giving researchers a single tool to support an integrated quality approach to optimise the availability of sample collections within population and research-based studies. Leveraging its ISIDOR™ platform, BioStudies provides advanced data visualisation that enables research organisations to elevate sample data from a commodity view into a more strategic, reusable and ultimately valuable scientific asset. The insight and analytics supported by BioStudies improves global sample data integration; creates real-time data visibility and access; enables the connection to bioprocessing data; tracks sample consent for sample usability; provides intuitive search and delivers custom data reporting. With BioStudies, researchers can quickly identify the right sample characteristics for medical research studies using integrated sample data analytics and request access to high-quality samples and data sets, which in turn reduces research costs and speeds medical therapies to market faster (**Figure 14**).

Many of today's current methods for cryopreservation of samples require chemicals and maintenance. Isopropanol (IPA) containers used for cryogenic freezing require costly alcohol replacements every five uses, can be cumbersome to handle and may have inconsistent freezing rates. As an alternative, Corning® (www.corning.com) CoolCell® is a reusable, alcohol-free freezing container, which controls the rate of freezing to -1˚C/minute when placed in a -80˚C freezer to uniformly freeze cells at a lower cost of use. The patent-pending CoolCell® technology utilises a thermo-conductive alloy core and highly insulative outer material to control the rate of heat removal and provide reproducible cell cryopreservation with high cell viability. In addition, Corning 1D/2D barcoded cryogenic vials allow for efficient management and manipulation of multiple storage tubes. Laser-etched 2D

Figure 13
Bluechiip-enabled products and services around in an OEM offering

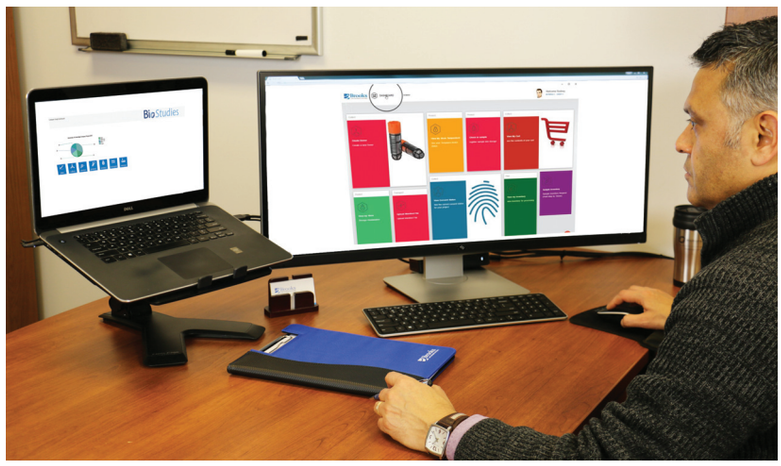

Figure 14
Brooks Life Sciences BioStudies® Biobank Data Management System – overcoming data integration challenges through a centralised technology platform

Figure 15
Corning® CoolCell® freezing containers use a combination of uniform-density cross-linked polyethylene foam, a solid state core, and radial vial symmetry to create freezing profiles that are consistent and reproducible (left). Corning® polypropylene cryogenic vials are available in a number of styles and can withstand temperatures to -196˚C (right)

barcodes on the bottom of the vial, as well as linear barcodes on the side of the vial, can be used for permanent sample identification, ensuring a reliable chain of custody. These cryogenic vials are temperature-resistant polypropylene, withstanding temperatures down to -196˚C, and are compatible with most scanning and capper/decapper systems. The company also offers cryogenic vial grippers featuring a unique gripper design that allows vials to be easily sorted or moved while maintaining sterility and protecting fingers from frozen vials, dry ice and liquid nitrogen (**Figure 15**).

Figure 16
LabElite I.D. Reader with ColdScan technology from Hamilton Storage

The LabElite I.D. Reader with ColdScan technology from **Hamilton Storage** (www.hamilton-storage.com) provides fast and accurate sample identification directly out of a freezer. This maintains sample integrity without compromise from the surrounding environmental temperature, and also prevents the risk of identification errors due to fog build-up on the scanning platform. Whereas passive anti-fog methods coat the scanning surface with an anti-fog spray which may lose effectiveness over time, ColdScan is an active and permanent method to combat fog and condensation on the barcode scanner. The I.D. Reader automatically decodes 2D barcoded tubes, with optional 1D barcode capability, on a wide variety of tube racks, including honeycomb-shaped racks. In addition to improving manual sample identification and tracking, LabElite I.D. Reader is an ideal complement to Hamilton Storage's robust and user-friendly automated sample management solutions, for further efficiency in sample storage, identification and tracking workflows. For small-scale sample storage, Hamilton Storage SAM HD offers +4˚C, -20˚C, -40˚C and -80˚C models, and capacity up to 60,000 tubes in standard racks, or 86,250 tubes using high-density RackWare® racks. Verso® is suitable for medium- to large-capacity storage needs, with temperatures from ambient to -20˚C, and processing rates up to 1,500 tubes/hour or 170 plates/hour. Finally, Hamilton Storage BiOS® is specifically designed to store sensitive biological samples at -80˚C, with capacity from 100,000 to more than 10 million samples, and support for a wide variety of labware (**Figure 16**).

Labcon North America (www.labcon.com), the world's leading manufacturer of Earth Friendly® laboratory consumables, has developed a new family of MEMS-enabled consumables initially targeted for cryogenic applications. Labcon is now offering these feature-rich consumables together with Bluechiip in a variety of form factors such as

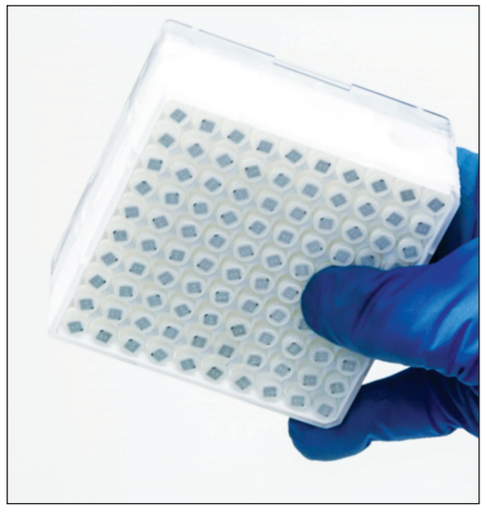

Figure 17
(Left panel) Labcon 2mL Bluechiip-enabled cryovials with 2D codes. (Right panel) Labcon's new 10x10, Multi-Vial Bluechiip reader

cryovials, centrifuge tubes and multi-well microplates. The first iteration of the technology enables Labcon to produce innovative, value-added, Bluechiip-enabled consumables that help sustain sample quality and chain of custody for both biobanking and compound storage customers. This leading-edge product line can track the ID of each sample, record all temperature excursions experienced during the lifecycle of each sample, and perform consistently at temperatures down to -200°C. Additional features include the ability to perform high-temperature sample tracking and auditing where temperatures can reach +100°C, such as in qPCR. Labcon also offers Bluechiip-enabled data tracking tools such as single-vial and multi-vial benchtop scanners, handheld mobile readers along with software that easily integrates into biobanking or compound storage systems. As researchers continue to develop new biological entities (NBEs) or advanced therapies derived from human cells, their efforts create samples that are extremely high in value, many are irreplaceable, and all of these constructs are very sensitive to temperature change. When Labcon Bluechiip-enabled consumables are deployed as a system, with their peripheral readers, transport appliances, and inventory software packages, they provide excellent custodial tracking and maintain the highest sample quality possible irrespective of the temperature excursions experienced by the samples (**Figure 17**).

Figure 18
Labcyte's 96-well acoustic tube and tube rack compatible with Echo liquid handlers

Labcyte (www.labcyte.com) in collaboration with Brooks Life Science Systems and AstraZeneca recently reported on its progress to develop a sample tube that will be compatible with its Echo® Acoustic Liquid Handlers at the 2017 Society for Laboratory Automation and Screening Conference. The adoption of Echo Liquid Handlers to transfer samples and reagents into assay plates for miniaturised high-throughput screening and lead optimisation has improved the efficiency and reliability of screening programmes throughout large pharma and life science research. These improvements are largely a result of the Echo system's ability to transfer nanolitre volumes of samples and reagents without the use of tips or contact of any kind. The development of a sample tube that is compatible with acoustic liquid handling will enable Echo system users to transfer samples into assay-ready plates without requiring the samples to be in a microplate first. The use of tips and plates just to prepare samples for transfer on Echo systems will be eliminated. This will also

Figure 19
Integrated STC7k5-ULT -80°°C storage system from Liconic Instruments which can be configured to incorporate a multi-vial rack MEMS reader

eliminate any risks from leachates or sample retention errors in tips that can lead to false positive or negative results from a screen. Additionally, the ability to reduce the amount of sample required to produce a screening result can enable the screening of rare compounds or compounds produced from low-yield synthesis reactions. With the launch of the new sample tube compatible with Echo liquid handlers planned for 2018, Labcyte intends to transform compound management and significantly improve the quality and reliability of screening programmes (**Figure 18**).

Liconic Instruments (www.liconic.com) is known worldwide as a leading manufacturer of automated plate, tube and vial stores for life science sample management. Liconic offers built 'fit-for-purpose' storage solutions addressing current applications in biobanking, cell-based drug screening, genomics, proteomics, diagnostics and precision medicine. The expanded use and application of biological-based materials for life science R&D and healthcare methods demands more critical scrutiny of their temperature sensitivities and more precise temperature control of those materials within a given application. More precise temperature tracking of individual samples is indicated. ID tracking within automated systems is a well-developed technique, the most popular current method employing 1D and 2D barcode labelling with optical reading. In addition to overcoming potential optical-based reading limitations such as interference from moisture (eg condensation, frost) and/or barcode flaws, wireless MEMS-based tracking technologies can precisely link the sample and its unique temperature history data. Currently, actual sample temperature in most applications is only inferred from temperature monitoring in the devices involved in any given process. The MEMS technology allows for direct, real-time interrogation at the level of the actual sample or the rack containing the sample. Liconic can now configure any of its storage systems to incorporate Bluechiip tracking technology – from small capacity, multi-climate option plate storage and incubators, to its larger-scale, application-rich, sample storage systems (room temperature down to -196°C). This can provide direct, unambiguous temperature data for a sample within a temporal process or over an archival storage period (**Figure 19**).

TTP Labtech (www.ttplabtech.com) is an innovative supplier of automated laboratory systems including storage, nL liquid handling and detection systems. TTP Labtechs offer modular, high-density storage systems in the product families of comPOUND and arktic, utilising 2D barcoded tubes for secure sample tracking with temperature options from ambient to -80°C. Tube racks are used for input and output of samples but not stored giving unrivalled storage capacity per m^2, rapid cherry picking and the avoidance of rack edge effects during freezing for improved sample integrity. Modular storage units offer simple scalability and a range of accessories providing future-

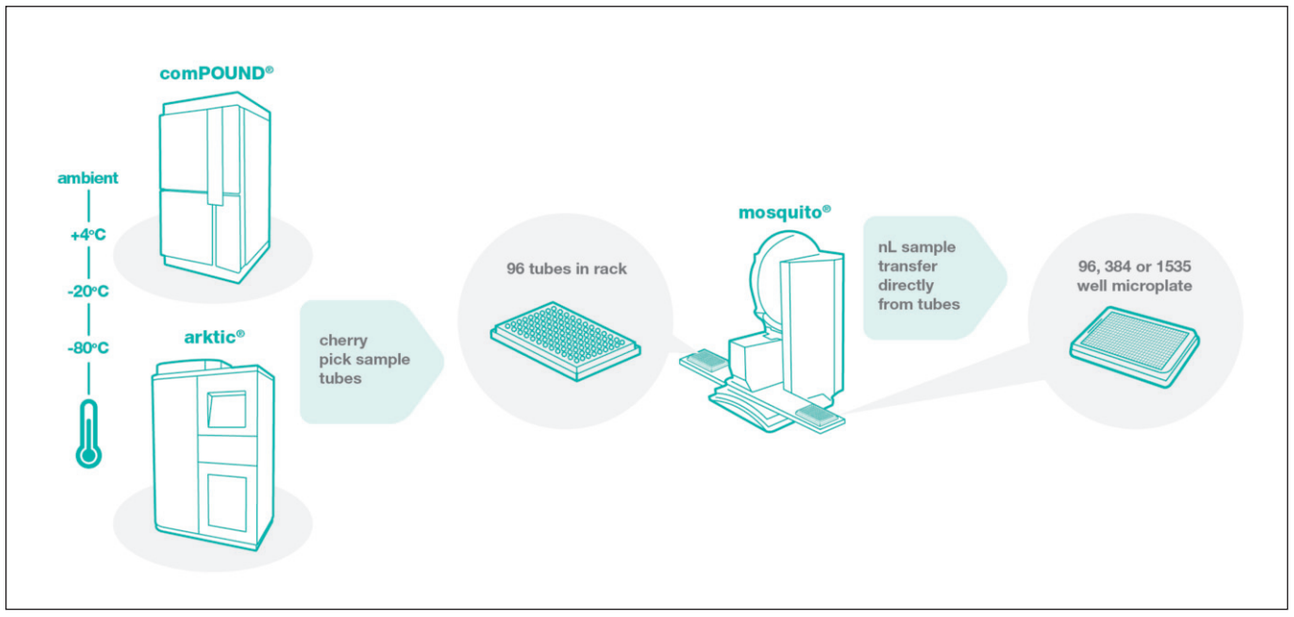

Figure 20: Samples can be cherry picked directly from TTP Labtech's comPOUND or arktic stores (at any temperature from ambient to -80°C), then aspirated directly from 2D storage tubes using mosquito into any density microplate (in nL-µL volumes)

proof paths to automation including the comPILER fully-automated storage tube to assay plate processing system. The storage systems employ unique pneumatic transport of storage tubes, providing industry-leading reliability by not requiring robotics in the cold zone. Recent developments have seen this transport system extended to transport storage tubes between labs and even buildings utilising TTP Labtech's lab2lab technology. Samples can be transported between a variety of devices such as stores and other integrated systems for direct analysis, such as HPLC, LC/MS and NMR. The latest development sees the marriage of low-volume 2D barcoded tubes with the TTP Labtech Storage systems and mosquito nL liquid handler. This combination enables mosquito to directly access the 2D storage tubes and prepare assay plates directly without the need for intermediates. This development offers enhanced efficiencies and significantly reduced dead volumes proving exciting possibilities for compound management, HTS, Synthetic Biology and a range of genomic applications (**Figure 20**).

Conclusions

There seems little doubt that preserving the quality of stored samples (both compounds and biospecimens) should be a high priority to those managing and end-users of compound management stores and biorepositories. What is less obvious is are there major innovations in available sample storage technologies that can identify poor sample quality or monitor sample degradation and so minimise erroneous downstream analytical results? Clearly recent advances in sample ID and tracking described above will undoubtedly help resolve problematic sample identification or misleading inventory info and give much greater confidence in maintaining a full audit trail. While new storage tube configurations will enable enhanced screening efficiencies, facilitate greater reliability and significantly reduce dead volumes. New cryopreservation technology also heralds more reproducible cell freezing with higher viability. In addition, advanced data management systems using integrated sample data analytics have the potential to identify the right sample characteristics for medical research studies. But do any of these technologies give reassurance that the stored samples are actually fit for their intended purpose and quell the crisis in end-user confidence in the quality of stored samples specifically associated with some biobanks? The Bluechiip MEMS-based sensor chip approach to temperature and ID tracking over the sample life cycle comes the closest, as potentially it could lead to stratification of samples into fit-for-purpose categories based on their previous temperature exposure history. However, tracking sample temperature lifecycle by itself will not improve on the quality of samples that were intrinsically of poor quality at the outset, and will only enhance the integrity among stored samples if there is willingness to act

upon the result of 'outside set range' temperature sensing (eg by removing and/or destroying such samples from the available collection). In an ideal world what is more needed are simple and fast QC/QA verification tests that can confirm a sample is what is supposed be or if it has suffered significant degradation. There are bioanalytical techniques such as the RIN score for RNA and the 260/280 test for nucleic acid quality assessment that have applicability to biospecimens, but their use in biobanks is currently limited. Some compound stores undertake mass spec confirmation of their samples, but extending the process to an entire legacy collection is a major undertaking. In addition, the availability of a gold standard technique that could enable universal QC/QA (eg biomarkers with predictable degradation profiles that could be used as surrogates of biospecimen integrity) currently appears to be a rather distant prospect. Until such tests become routine, storage facilities and end-users of stored samples need to do all they can to try to maintain sample integrity. This includes: strict adherence to protocol during sample preparation prior to storage to minimise pre-analytical variables; increased awareness (better training) in standardised biosample collection techniques; undertake sample verification prior to storage (where feasible); eliminate all freeze-thaw cycles; minimise hydration; uphold an unbroken chain of custody (audit trail); monitor storage temperature (where appropriate and feasible); and to be prepared to take the decision to cull samples of suspected low quality, that are partially degraded or of uncertain authenticity/history from existing inventories. DDW

Reference

1 Sample Storage Quality & Identification Tracking Trends 2016. Published by HTStec Limited, Godalming, UK, October 2016.

Dr John Comley *is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation; assay methodologies and reagent offerings) to drug discovery and the life sciences. Since its formation in 2002, HTStec has published 135 market reports on enabling technologies and Dr Comley has authored 62 review articles in* **Drug Discovery World**. *Please contact info@htstec.com for more information about HTStec reports.*