

## OUTSOURCING

# Sourcing clinical patient samples for IVD development

A review of current issues encountered when sourcing blood-based clinical patient samples and the future outlook for IVD manufacturers. **BY SIMON PACKER, KELI STOCKBRIDGE, AND PHILIP JEWESS**

**C**linical patient samples are an essential but often overlooked tool in numerous clinical and medical applications. In the IVD industry, they are widely used during the various stages of test kit development, including marker discovery, field trials, product validation, and preparation for regulatory approval. They are essential to ensure the scientific relevance and specificity in the clinical application of a novel IVD test or instrument before it can be launched into the marketplace. Clinical patient samples can include any body fluid ranging from saliva to cerebrospinal fluid. However, blood-based samples such as serum and plasma are by far the most common types of samples used for developing commercial IVD tests.

It was not until 1976 that FDA differentiated between medical devices and drugs, and an elaborate and detailed scheme to regulate medical devices was established in the United States. Legislation passed in 1976 dictated that simple clinical patient samples with a measured analyte level were sufficient



Automatic sampling systems allow for efficient storage and handling of a diverse range of clinical samples.

during product validation. Since then, FDA requirements have been modified numerous times. However, unless a device has been identified for premarket approval (PMA) or special controls have been requested by FDA, very little premarket analysis is required. Figure 1 highlights the key modifications to FDA legislation during the past 80 years.

The recent report submitted to FDA by the Institute of Medicine (IOM), “Medical Devices and the Public’s Health: The FDA Clearance Process at 35 years,” has recommended that an integrated premarket and postmarket regulatory framework should be introduced. If such a framework is implemented, it would enhance the demands

1938 Act		1976 Act		1990 Act		1997 Act		2002/07 Act	
<ul style="list-style-type: none"> <li>• Introduction of medical device regulation</li> <li>• Medical devices regulated under the same system as drugs</li> <li>• Strict pre-market reviews for numerous devices</li> </ul>		<ul style="list-style-type: none"> <li>• Medical devices differentiated from drugs</li> <li>• Pre-market Approval (PMA) limited to fewer devices</li> <li>• Introduction of device classes (Class I, Class II, Class III)</li> </ul>		<ul style="list-style-type: none"> <li>• Introduction of class reclassification procedures</li> <li>• Introduction of 'Special Controls' for Class II devices</li> <li>• New post-marketing tools introduced</li> <li>• Publication of the requirements for PMA submission</li> </ul>		<ul style="list-style-type: none"> <li>• 510(k) notification no longer required for Class I and some Class II devices</li> <li>• Reporting requirements relaxed</li> </ul>		<ul style="list-style-type: none"> <li>• Introduction of user fees to expand pre-market review capabilities</li> <li>• Performance goals introduction for FDA</li> </ul>	
1930	1940	1950	1960	1970	1980	1990	2000	2010	

**Figure 1.** FDA legislation developments during the past 80 years.

on IVD manufacturers to show evidence of a product's safety and effectiveness before it is launched onto the marketplace. IVD industry developments, such as companion diagnostics and the requirements for evidence-based medicine, are also fuelling this demand for improved premarket analysis. Inevitably, this will affect the amount of resources IVD manufacturers will have to dedicate to sourcing clinical patient samples to meet these requirements in the future.

For IVD manufacturers, sourcing high-quality blood-based clinical samples can often be problematic. Numerous considerations have to be taken into account when selecting a cohort of samples to validate a novel IVD test, ranging from the pre-analytical status to ensuring a back-up supply for future work.

## Using Clinical Samples and Their Requirements

Developing an IVD test can take many years, from the initial biomarker discovery to a successful commercial launch. During the complete development process, clinical patient samples play a key role. In the marker discovery and initial research phase, small quantities of samples with a clinical diagnosis or elevated analyte levels are required to demonstrate proof of concept. Such samples tend to require minimal patient information and are generally fairly simple to

source. At this stage, if samples are too difficult to source, insufficiently robust, or not representative of the final application, the IVD cannot be developed any further for day-to-day clinical use.

If the research stage proves successful, lab and field trials can be initiated. At this point, the requirements for the clinical patient samples become more tightly defined, and more time and resources have to be allocated. Currently in the United States, all new IVD tests must be registered with FDA and fall into one of the three medical device classes detailed below. This classification will define the premarket testing procedures and clinical evidence required for that particular device.

- Class I: A device of which the general postmarketing controls would be sufficient to provide reasonable assurance of safety and effectiveness.
- Class II: A device which cannot be placed into Class I because the general controls are not sufficient by themselves to provide reasonable assurance of safety and effectiveness, but on which there is sufficient information to establish a performance standard to provide reasonable assurance.
- Class III: A device that is represented as being for use in supporting or sustaining life (or in

preventing impairment of health) or that creates a potential unreasonable risk of illness or injury and that cannot be placed into Class I or Class II.

For Class I and Class II devices, an IVD manufacturer is usually required to submit its diagnostic test for 510(k) premarket notification before being launched on the market. For Class III devices, the manufacturer is required to submit a PMA application. All three classes require the manufacturer to submit data that demonstrates clinical performance compared to existing products on the market.

If the IVD is targeting a novel biomarker, and there is no direct comparison method on the market, it will likely be classified as Class III, or alternatively Class II with an FDA request for special controls. In this case, a larger amount of supporting data may be required to demonstrate the test kit's clinical effectiveness. Such data require a large number of clinical samples with in-depth patient information in order to establish an evidence-based assessment of clinical utility.

## Current Issues in Sourcing Samples

IVD manufacturers have at their disposal two widely used methods to source clinical patient samples: commercial outlets and direct collection centers. Whichever supply route

a manufacturer chooses, both types of suppliers will be faced with some common issues discussed below.

### Reference Method

When deciding which cohort of patient samples to use, one of the first considerations is the selection of the reference method. Choosing the reference method for comparing a novel test is vital to establishing the validity of the data produced. If there is an internationally accepted reference method (e.g., the peptide digest method used in the IFCC standardization of the HbA1c standard), the method used for comparison should be directly traceable to it. In some instances, there will be no such accepted reference method, in which case, the investigator must use the clinical information to judge the most relevant comparative diagnostic criterion.

### Biomarker Values

The values of the biomarker in question in the sample cohort are important in selecting a relevant test sample panel. The range should reflect the biomarker values that clinicians will be looking for when ordering a test for that specific marker. Hence, the sample panel should include samples in or around the reference range, and up to the assay cut-off point for a positive diagnosis. The panel should also include samples that go from the minimum detectable biomarker value (sensitivity challenge) up to the highest biomarker levels. Within the panel, the number of samples at each biomarker level should reflect the patient ratios expected in the relevant testing populations.

### Reference Range

Establishing a reference range for an assay is often done after introducing the assay at a local level, which is still a very important exercise undertaken by individual testing laboratories. However, when

validating a test kit, it is important to conduct sufficient testing of a normal population defined from the testing criteria and for potential differences in the samples' clinical information, which will provide a baseline for the IVD test. Racial, geographic, and dietary differences may affect the reference range, and clinical cut-off levels should be established as soon as possible.

### Test Interferences

All IVD tests are potentially susceptible to test interferences, which should be assessed within the relevant sample cohort. The potential interference samples used should reflect the likely interference factors within the testing population and should include common interfering substances such as hemolytic and lipemic samples. Specific rare interfering factors such as HAMA and closely related cross-reacting biomarkers should also be considered. Such interfering factors should ideally be native samples with varying levels of the target biomarker to challenge the testing method's performance and specificity.

### Pre-Analytical Status

When carrying out in-depth 510(k) reviews or submitting PMAs, IVD manufacturers should consider the pre-analytical status of the clinical sample. To obtain the best results, it is essential that all test material has been handled in a consistent manner appropriate to maintaining sample integrity, which includes collection methods, time-to-freezing, and sample storage. Information on these aspects of the sample can often be extremely difficult to obtain since they are not standardized from hospital to hospital. Unless specific requirements are known at the early collection stage, it is nearly impossible to control.

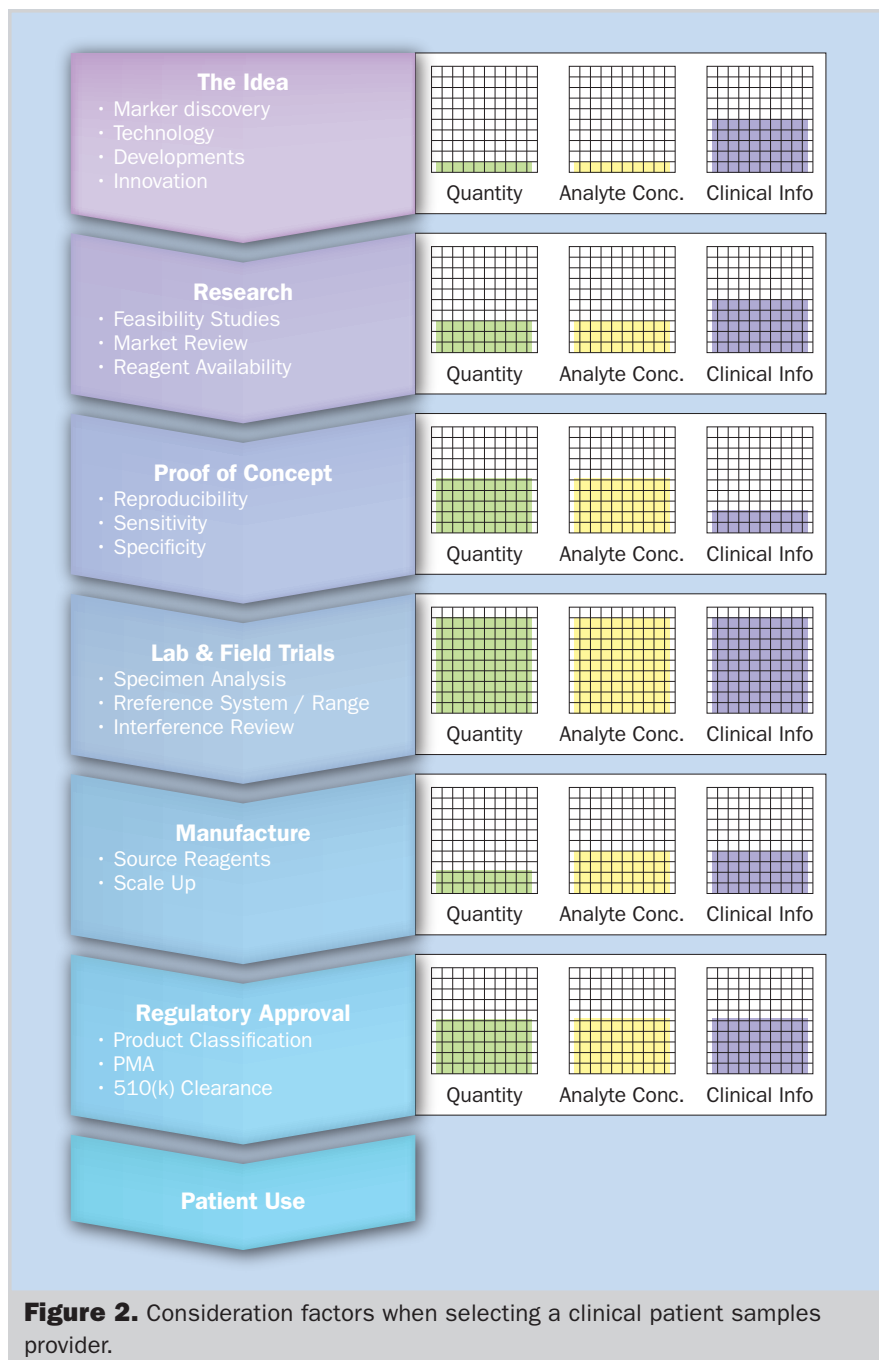
An example of where control of the pre-analytical status of samples is important is in commercial Tro-

ponin I assays. Falsely elevated troponin I levels are observed if fibrin is found in the sample. Fibrin formation can occur from inadequate tube mixing on collection, inadequate clotting time, and cold activation of clotting factors. When it is possible, IVD manufacturers should follow a standardized sample collection protocol that defines all of the key variables from the preanalytical phase (e.g., time and method of specimen collection, storage at every stage) to the analytical phase and the time taken from sample collection to sample analysis.

### Tracking, Stability, and Quality Assurance

IVD manufacturers should also consider storage, relative stability, and shelf-life of different sample types when utilizing clinical patient samples. When a large number of samples are required but will be used over an extended period of time, this raises the issue of where and how they will be stored, especially if they are being stored by the end user. Many sample suppliers can store clinical samples in their biorepositories and then send them to the manufacturer as needed. Manufacturers must assess the sample suppliers to ensure they have robust systems in place for tracking patient sample collection and for guaranteeing the continuity of the sample supply. The storage system used for the samples should be relevant for the sample and monitored to assure the sample integrity.

Another important factor to consider is the accuracy of the clinical data and ensuring that the data is relevant to the samples provided and viable for direct correlation to the final IVD kit application. Using a biorepository or supplier with a proven track record, a well-defined quality system, and well-established standard operating procedures is one way of ensuring the samples used will have the integrity



required for most regulatory bodies. IVD manufacturers can monitor the integrity of the specimens stored at a biorepository by including aliquots of some specimens in the repository, which have been tested at the same laboratory responsible for generating the data that will be submitted to gain approval of the biomarker for clinical use. The aliquots act as controls that are stored on site and are analyzed at the time of specimen testing.

## Clinical Information

Another pressing issue for IVD kit manufacturers is ensuring all the relevant clinical data required are available for a patient sample, which is especially true as the demand for evidence-based medicine becomes more prevalent. In this instance, there must be evidence to demonstrate how an IVD kit will actually assist in diagnosis and treatment, leading to a positive outcome. To achieve this, having the clinical

sample's relevant patient information such as age, sex, demographics, disease prognosis, clinical stage, current and past treatments, treatment response, and any other relevant medical history is essential to produce accurate testing data. Such information can often be difficult to obtain, especially as the majority of samples that are readily available in the marketplace are left over from hospital pathology tests, without any key patient information being made available to the end user.

Sourcing a diverse range of clinical samples can often be difficult if the relevant marker is specific to one particular demographic or global region. Samples used to assess a diagnostic marker should ultimately reflect the population that the marker will be used to test. However, the original testing should also assess diagnostic accuracy in as wide a range as possible, and therefore the cohort of samples should include samples from every demographic, age, and socio-economic group. Samples should also be related to the stages of disease. For example, in the case of tumor markers, samples can reflect early tumor development, each stage of chemotherapy, and benign and highly aggressive tumors.

## Ethical Considerations

It is critical to ensure that Institutional Review Board (IRB) approval has been achieved and that evidence of patient consent for a specific end use is made available to the IVD manufacturer. This factor is especially important since FDA liability for this rests with the manufacturer rather than the sample provider. Evidence of consent may be required during an FDA audit, making it an essential piece of information. Post-approval launches of kits to market may also prompt requests from potential customers to demonstrate compliance with ethical sample collection procedures.

## Sourcing Clinical Patient Samples

Before sourcing a selection of clinical samples, one of the most important steps is to define clearly the individual requirements. Such requirements can include the following: the number of samples, analyte levels required, patient information, and evidence of patient consent.

Defining the required timeline at this early development stage is important in order to ensure no costly delays in the process. Realistic timelines for sourcing a bulk number of clinical samples can vary depending on the specific requirements, but they should be established at the outset of any project. For commonly occurring conditions in developed countries (e.g., prostate cancer or kidney failure), many commercial outlets have stocks of patient samples readily available, which could be processed in as little as 2-3 weeks. For rarer conditions, and where extensive patient information is required, the collection times can extend from six months up to a year to obtain a diverse range of samples. It is essential that the samples are stored and handled correctly during these extended periods to ensure that no stability issues are encountered.

Once the sample requirements are clearly defined by the IVD manufacturer, the next step is to select the most appropriate supplier. In most cases, a commercial supplier is selected since they will have a large biorepository along with a number of sources, allowing for a good collection rate and scope to accommodate varying specifications. Alternatively, manufacturers can set up agreements with direct collection centers and hospitals. This approach can be effective when only routine samples are required and can sometimes be more cost effective. However, the scope of individual hospitals is localized and limited, and dealing with them can be time-

consuming and resource-heavy in the initial set-up stages.

Once an IVD manufacturer has selected a sample supplier, other key factors need to be considered to make sure all requirements are met, including price, collection completion timescales, standardized collection procedures, supplier quality systems, and customer service. If a manufacturer uses a commercial supplier, establishing a supplier agreement to ensure a continuous supply of samples within an allocated timeframe can be beneficial. Figure 2 shows the stages of test development, along with the sample requirements and key factors to consider when selecting a supplier.

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### Future Outlook

In the future, IVD manufacturers could face a number of potential changes when sourcing clinical patient samples. With greater demands for more specific and accurate tests, as well as the continuing emergence of companion diagnostics and multiplex assays, there is an ever growing demand for clinical patient samples with better and more complete patient information than ever before.

The recent IOM report concluded that the current 510(k) clearance process for Class I and Class II devices was not designed to evaluate a medical device's safety and effectiveness, but rather only assesses its similarity to commercially available devices prior to 1976. The report recommended

that an integrated premarket and postmarket regulatory framework be introduced to replace the 510(k) clearance process. If such a framework were implemented, it would increase the requirements for IVD manufacturers to show evidence of a product's safety and effectiveness before being launched on the marketplace, which would require large volumes of clinical samples with relevant collection controls in place.

In the IVD market, demands for IVD tests with greater accuracy and specificity for elements such as disease progression and prognosis are increasing. Key research institutes are developing a wide variety of novel markers that could provide a vast range of possibilities for the IVD market. Once a specific marker has been selected for further development and commercial launch, generating evidence of its relevance is essential.

One example is recent research into the use of PSA isoforms as a more accurate diagnosis of prostate cancer, thereby reducing the number of biopsies required. Initial research has shown that PSA isoforms provide a higher predictive value compared to traditional PSA tests. However, they do not give an indication about the aggressiveness of the cancer. If the tests for specific isoforms were to be developed into a marketable device, an in-depth clinical study would be required, and a large range of clinical patient samples with vast amounts of associated data would be needed. This places significant development and financial demands on IVD companies, and restricts the desire to develop novel but clinically desirable devices. During the next 5-10 years, the demand for clinical samples with greater patient information is expected to increase rapidly and is likely to be one of the most significant changes when sourcing samples in the future.

Other IVD advances will also affect clinical patient sample

requirements. Multiplex assays are now more widely used than ever before, prompted by the completion of the human genome project, with growth likely to continue during the coming years. Since this type of assay looks at hundreds of markers simultaneously, large and diverse ranges of samples with a selection of marker levels for each measured analyte are required during the validation phases. The pre-analytical challenges for each analyte in multiplex assays also make their approval more problematic. Sourcing this type of sample variety will require more investment and resources during the validation stages of development.

One of the key developments expected during the next 5–10 years is the collaboration between the IVD and pharmaceutical industries to develop companion diagnostics. Although companion diagnostics have seen a slow start, a few success stories have meant that strategic growth in this area is likely to continue. With the collaborations between pharmaceutical and IVD companies, enhancing the product analysis and generating medicine-based data associated with both the diagnostic test and the companion drug are essential. The regulatory

demands on the pharmaceutical and biotechnology industries are very significant. IVD companies will likely have to elevate their abilities to meet expectations and regulatory requirements, so more samples will be required to provide extensive statistically relevant data.

In tandem with high-end kits, there is also a significant demand for developing simple, cost-effective IVD tests suitable for developing third-world countries. As these countries advance economically, this trend is likely to continue in the future. When developing and validating such tests, one of the most common issues is sourcing good clinical patient samples. Often the diseases that are tested in third-world nations only occur regularly in a specific demographic area. They are predominantly infectious diseases such as malaria and HIV, along with diseases that are now rarely seen in developed countries such as polio and tuberculosis. As demand for IVD tests increases in these developing countries, more economical solutions will reach the market. This means greater demands for clinical patient samples to validate these economical alternatives and higher investments in establishing a suitable supply chain

for appropriate samples.

The future for IVD manufacturers will hinge upon legislation-related changes and scientific advances in the industry. If the recommendation to FDA for an integrated premarket and postmarket regulatory framework for medical devices is implemented, clinical sample requirements will likely be amplified in the future. Moreover, with the development of new technologies, novel biomarkers, multiplex assays, and companion diagnostics, IVD manufacturers will have to expand the time and resources allocated for sourcing clinical patient samples. [IVD](#)



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