Microfluidics for IVD analysis: Triumphs and hurdles of centrifugal platforms

Part 3: Challenges and solutions

This article addresses challenges that remain to developing a microfluidic nucleic acid diagnostics system, with solutions and a description of a forward-looking system. BY JONATHAN SIEGRIST, RÉGIS PEYTAVI, MICHEL BERGERON, AND MARC MADOU

The ideal microfluidic nucleic acid (NA) IVD device should be as simple to manufacture and use as currently available handheld glucometers. Point-of-care (POC) glucometers can be operated with minimal training, cost less than $100 to manufacture, are powered with small, standard batteries, and require disposables that are inexpensive to produce. However, such an equivalent system for NA testing does not exist today because NA assays are much more complex (see Figure 1).

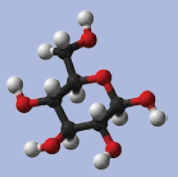
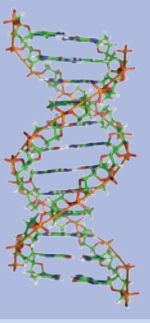
	Glucose	Nucleic Acid
Analyte		
Location	Free in blood/ serum	Enclosed in cells/ viruses
Concentration	mM	pM or even less
Identification	Detection if present	Detection of specific sequences

Figure 1. Comparison between point-of-care detection of glucose and nucleic acids. Glucose detection is a relatively simple process since fewer restraints are placed on the molecule of interest compared with nucleic acids.

As discussed in part one of this article (*IVD Technology*, November/December 2009), NA assays require procedures such as lysis, purification and concentration, amplification, labeling, and specific sequence detection. Naturally, these well-established yet complex procedures that are conducted on benchtop equipment were the first to be adopted into micro total analysis systems (microTAS) for IVDs. Such adoption has led to complex, high-cost systems that are a far cry from a simple glucometer. To be successful, microfluidic IVD systems have to be redesigned from the start as simple, integrated instruments with disposable cartridges that are capable of low-cost, low-complexity, and low-power operation. To achieve this objective, innovative strategies in terms of design, manufacturing, reagent storage, and power use have to be developed, which reflect biological needs and constraints.

Parts one and two (January/February 2010) gave an overview of the important biological and engineering issues related to microfluidic NA diagnostics. This final article discusses the remaining challenges to developing a microfluidic NA system for IVDs by offering some solutions and describing a forward-looking system that can be used both in and out of clinical settings. Although this article places an emphasis on POC devices, many common challenges are presented for both laboratory and POC systems.

Design and Manufacturability

Complex biological assays can be very sensitive to changes in the environment in which they are run. The biological steps required for microTAS systems in the future will need to be redesigned to be robust, simple,

and far less sensitive to environmental changes. Expecting a microTAS system to deliver perfectly pure samples to downstream steps (e.g., amplification and detection) every time is unrealistic. Moreover, the purity of the samples delivered for these steps will vary, depending in part on initial sample characteristics. Thus, the steps downstream from sample preparation must be designed to be robust, even for nonpure, dirty solutions.

Optimizing biological approaches can help to reduce the required sample size. For conditions such as sepsis, the microbial load can be very low, requiring a large sample volume for detection, as discussed in part one. However, this requirement is true only if the presence of the pathogen NA is directly detected. Alternatively, detection techniques could focus on the host's response to the pathogen, which would use detectable analytes that occur in much higher concentrations. Such an assay would require much less sample volume and possibly avoid an amplification step.

To lessen the device's biological and microfluidic complexity, it should combine as many assay steps as possible. For example, performing lysis in the presence of beads functionalized with robust capture probes could combine the lysis and concentration/purification steps. Real-time PCR is an excellent example in which two steps (amplification and detection) have been joined together into one step. NA amplification could also be run in the presence of capture beads, eliminating the need for a separate, dedicated microfluidic chamber for amplification. Tradeoffs and compromises between the assay and the microfluidics must be made.

The microfluidics of the device itself must also remain simple. To that end, the total number of chambers, channels, and valves required must be minimized. Another significant source of complexity comes from placing various materials inside microfluidic devices for added functionality. As described in part two, examples of such materials include magnets, beads, and wax. Although the use of these materials should be minimized, they are not likely to be completely eliminated since they are a convenient if not necessary way of adding functionality.

In order to make the microfluidic devices economically feasible, they must be mass manufactured. Silicone materials have become well-established for fabricating microfluidic devices for laboratories. However, many of these silicone materials have severe limitations for the applications discussed in these articles, such as small-molecule absorption and permeability to water vapor. The limitations prevent such microfluidic devices from being used for longer-term applications outside of the research laboratory. (Shelf-life for a commercial product must be more than six months.)[1]

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In addition, the truly microscale features enabled by soft-lithography and microfabrication may not be required. Future microTAS platforms must be able to handle volumes in the µL-mL range. This volume requirement translates to fluidic conduits ranging in size from hundreds of µm to mm. Since micro and nanoscale features designed to handle nanoliter volumes and less may not be necessary, soft-lithography and silicone-based microfabrication techniques are not viable manufacturing candidates for producing microfluidic disposables.

Today, few manufacturing techniques are as economically viable as injection molding of plastics. Injection molding technologies have made many advances and can currently create microscale features using a wide array of materials with the tolerances required for repeatable performance of the microfluidic device. This manufacturing technology will be used to make microTAS devices in the future. However, injection molding presents some unique challenges since the device must contain macro- and micro-scale features on the same plastic part.

The injection molding methods must exhibit a large dynamic range of manufacturable sizes in order to deal with the widely varying dimensions and scales. In order to reduce complexity, the microfluidic device should also be made of as few different materials as possible. It should ideally be a monolithic structure made of only one material. But the device more realistically will be made of at least 2-3 parts, and some parts may require bonding via thermal, chemical, or ultrasonic means.

Bonding of different parts is an important yet often underestimated manufacturing step. The injection-molded microfluidic devices will initially have exposed channels open for inserting materials and reagents. A featureless cover layer of plastic will be applied to close and seal the device. At this point, the device will have sensitive components onboard (e.g., reagents) and perhaps surface treatments. If chemical bonding is used, residues cannot be left behind that may interfere with the analysis. If thermal bonding is used, the temperature may not exceed 100°C since it will inactivate any enzymes and other reagents onboard. Finally, the bonding method must not disrupt or distort any of the microfluidic channels, which can cause assay failure.

Liquid Storage and Lyophilization

While the manufacturing process should be kept as simple as possible,

the one unavoidable complication is the introduction and storage of reagents in the microfluidic device. A collection of different reagents such as NA probes, reaction buffers/salts, enzymes/polymerases, etc., will be required for the microTAS device to function as a standalone unit. But having such reagents manually loaded would increase the complexity of use for the device, so most if not all of the reagents must be stored on the device itself.

Since microfluidic devices must have a shelf life of at least 6-12 months, the reagents required need to be preserved on the device for that same period of time. In addition, the preserved reagents must be able to withstand a range of temperatures that could occur during shipment and storage of the device. A common and well-accepted method of reagent storage is lyophilization (i.e., freeze-drying) in which reagents are formed into powders that reconstitute when they come into contact with liquids. But it is also essential that the device contain its own liquid reagents, since most of the assay steps occur in an aqueous form. Relying on users to introduce outside water invites a huge source of variability since using water from unclean, contaminated sources could cause assay failure.

Liquid reagents can be stored long-term in glass ampoules or metalized plastic pouches. Such pouches can be pierced or opened with physical pressure by users or a mechanical actuator that is part of the instrumentation. Packets or pods of liquid can be manufactured independent of the microfluidic device, and then included with the device during fabrication.

Environmental and Power Requirements

In order to meet operational requirements outside of clinical settings, the microfluidic IVD instrument must run on low power and must be unaffected by environmental conditions such as heat, humidity, and dirt. Currently, NA amplification methods such as PCR are the most power demanding and lengthy processing steps. For example, researchers have developed a microfluidic PCR card system (see part two of this article) that uses more than 150 Watt-hours of energy to power the Peltier thermocyclers and ice-valves during a standard PCR run.

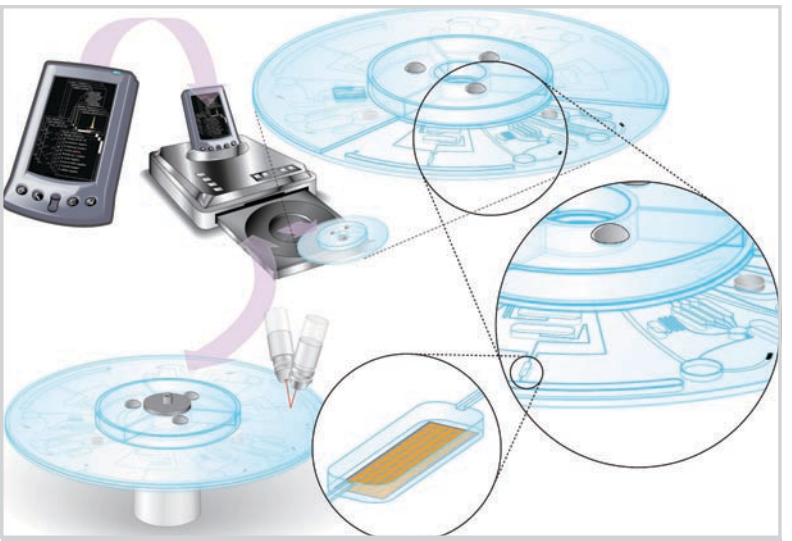

Figure 2. An artist's rendition of a complete CD system for nucleic acid IVDs, showing the disposable (containing a center centrifugal hub for large-volume sample preparation), optical detection via microarray, and the benchtop hardware system with a coupled handheld device.

However, NA amplification needs to move away from such high-power methods toward more-robust, low-temperature, isothermal schemes. Moreover, a more robust amplification method needs to be developed that does not amplify the NA directly but rather another reporter molecule. While such a new method would still rely on hybridization and/or polymerase extension events for initiation, the result would be amplification of a reporter that is much easier to detect without using large amounts of power.

Different heating methods must be examined for microfluidic IVD applications. While thermoelectric heating can provide precise control and rapid temperature changes, it requires intimate contact with the microfluidic device and large amounts of power. Infrared heating is an excellent alternative that can provide good precision without requiring contact. Chemical heating by use of exothermic reactions requires no external power, but it can be slow and difficult to control.

Thermal management in the microfluidic device must also be considered. The NA assay steps (e.g., hybridization) can rely on specificity by being conducted at a constant, usually slightly elevated temperature (e.g., 37°C). The hardware inside the platform (e.g., circuitry, light sources, and detectors) will also require the temperature to remain constant within a working range. Keeping the temperature constant will entail large amounts of power, since a platform functioning in the tropics where temperatures can reach 44°C or higher must be cooled. Such thermal management requirements are in addition to those for specific heating and cooling steps for the assays, which further adds to the system's complexity.

In addition, the microfluidic platform must not be affected by environmental factors such as dirt and humidity. Along with temperature, such common factors rarely have to be dealt with in laboratory settings. However, they can greatly affect the performance of amplification enzymes or the specificity of DNA capture probes. Ignoring such real-world factors will lead to a system limited

to use only inside well-controlled laboratories, and prevent the system from being applied where it is needed most, such as in developing countries.

CD-Based Centrifugal Microfluidics

The previous discussion provided a clear explanation of the many hurdles that must be overcome to develop a truly integrated micro-TAS system for NA diagnostics. Considering the advantages that centrifugal platforms offer compared with standard microfluidic devices (as discussed in part two of this article), POC microfluidic devices in the future are going to be based on the compact disc (CD) format (see Figure 2). With this format, most of the reagents are lyophilized and placed inside the CD during manufacturing, while the remaining reagents are in liquid form and stored in metalized pouches. While the CD itself is stored in a vacuum-sealed pouch impermeable to outside conditions, it does not require refrigeration for storage.

The CD platform is a larger-sized handheld instrument that is similar in size to a portable radio, and it is able to process a minimum of three samples per CD. The platform also includes a handheld wireless device that stores all test results and information. When a wired or wireless connection is available, the device uploads the results to a server, which allows an organized database of information to track the progression of diseases and possible outbreaks.

The CD device is injection molded out of a single type of plastic: polycarbonate. It consists of the following three plastic parts: a monolithic CD containing the fluidics for amplification and detection in the middle layer, a solid featureless bottom disc that is attached using thermal bonding, and one of many various CD sample preparation hubs placed on top. As has been emphasized, the largest source of variability in NA diagnostic processing comes from the different types of samples being processed. Thus, having various centrifugal sample preparation modules for different samples is essential.

Modular sample preparation hubs are designed for different sample types. For example, blood samples require hubs capable of processing larger volumes, and respiratory samples require smaller hubs. Such sample preparation hubs can be snapped into standard CD bases designed for amplification and detection to create the complete centrifugal microfluidic device. While this may be done during manufacturing, end users may opt to select on their own the appropriate sample preparation hub in order to retain optimal flexibility and modularity.

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The microfluidic system prepares samples using a combination of chemical/thermal lysis and light, low-power mechanical agitation. While the concentration and purification steps are performed using solid-phase extraction, they are combined with lysis to reduce the device's complexity. The system conducts amplification using a low-temperature, isothermal method. For steps including isothermal amplification and hybridization, heating on the platform is performed using a small infrared lamp in combination with exothermic chemical heating. A field-deployable microTAS system for NA diagnostics does not scan for only a few analytes, but rather a large panel of analytes. Thus, detection is performed using fluorescent DNA microarrays using low-power, inexpensive optical hardware (i.e., LED or laser-diode light sources and photodiode detectors).

Using respiratory samples as an example, the operation of the CD platform is as follows: the user puts a fresh battery into the bottom of the platform, selects an appropriate sample preparation CD hub for the respiratory samples, and attaches it to the standard amplification and detection disposable CD base; the respiratory samples from three patients are added to the hub, and the hub is closed; the user manually pushes three buttons on the sample prep hub that pierce internal metal pouches, thus releasing the stored liquid reagents; the CD is loaded into the platform, the appropriate program is verified from the interface screen, and the test is started.

During this operation, the samples are lysed, clarified, purified, and sent to the amplification chamber. These steps take 5-10 minutes and require a small amount of power. Amplification takes 30-35 minutes and is still the largest user of power. The samples then flow across the microarray, which takes ten minutes. Finally, the detection optics scan the array and upload the image for the diagnosis software. The test results are output to a screen in an easy-to-interpret, digital diagnostic format for each of the analytes. The CD is ejected from the platform and discarded by the user. The CD exits the platform with all ports and openings permanently sealed, making the risk of contamination and infection later on very low. The entire analysis takes less than one hour.

Much effort has been put into simplifying and combining the assay steps, and minimizing the power requirements in the CD platform. Although improvements still need to be made, the user experience is easy and streamlined, the test results are robust and reliable, and the CD disposable parts are inexpensive. The platform's success relies on the inherent advantages of microfluidic

centrifugal platforms and a cohesive design methodology, which keeps all aspects of the system in mind during development. The CD-based microfluidic IVD device presented is well adapted to addressing POC diagnosis and specification of panels of diseases showing common symptoms. For example, the system could distinguish between various types of influenza, or could be used for identifying the particular microbes involved in a case of sepsis.

Conclusion

The three parts of this article have stressed that while barriers to commercializing microfluidic NA diagnostic systems remain, many of them can be overcome. First, closer attention must be paid to the characteristics of the samples being processed, a subject that is often overlooked. Variations in the samples' surface energy, viscosity, and homogeneity can drastically affect both microfluidic and biological parameters. As discussed in part one, biological samples from ill patients can exhibit extreme variations of such characteristics, which further adds to the challenge. Sample volume must also be carefully considered such that a statistically relevant number of analyte molecules are collected for downstream steps (e.g., amplification and detection). The microfluidic device must be able to handle larger sample volumes on the order of several mL.

A second factor to overcome commercialization barriers is clarifying the differing jargon between biological and engineering disciplines. To this end, the articles have attempted to provide a clear view on some important issues and confusions that affect both disciplines. For example, when characterizing NA amplification reactions, engineers are rarely interested in knowing only how many initial analyte DNA molecules are present in a reaction tube. It is also essential to describe the volume conditions so that the starting concentration can be known. Similarly, biologists have little interest in knowing that a thermocycling system can heat and cool at record speeds. They are more interested in knowing the quality of the PCR reaction that results when using the microfluidic system (i.e., what is the lower limit of detection, and how reproducible is it?).

Third, the entire microfluidic system must be designed to be simple, with as few different materials, technologies, and number of parts as possible. The disposable CD must remain inexpensive, and the instrument must be able to handle all conditions, both in and outside the clinical lab. Thermal and power management will be a key component, and the system's environmental impact must also be considered.

A fourth factor to engender faster commercialization is to prototype devices using technologies that have a realistic chance of being transferred and scaled-up to mass manufacturing. This method is the only way to make the microfluidic devices economically viable and the entire system successful. Moving away from standard silicon micromachining and soft-lithography techniques is essential. The devices must be prototyped with technologies and methods that can be transferred to a plastic injection-molding method.

Many microfluidic technologies are available as candidates for creating a successful NA diagnostic device. Centrifugal platforms in particular hold some unique advantages, including low-power pumping, robust valving schemes, and ease of parallel processing. Several commercialization barriers have already been overcome in the development of CD systems for microfluidic NA diagnostics. The CD platform has matured to a point where real-world application is feasible, and no major technical obstacles remain. Defining a clear market application and a cohesive development plan will ensure success of a microfluidic IVD CD platform.

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Reference

1. MW Toepke, "PDMS Absorption of Small Molecules and Consequences in Microfluidic Applications," *Lab on a Chip* 6, no. 12 (2006): 1484-1486. IVD

Jonathan Siegrist is a recent graduate of the biomedical engineering department at UC Irvine. His areas of expertise include microfluidic devices for NA IVD, including standalone and integrated CD systems for sample prep, amplification, and detection. He is also a cofounder of iGlyko Inc., a medical device firm.

Regis Péytavi, PhD, is a project leader at the Centre de Recherche en Infectiologie of Université Laval in Québec City, and the technological coordinator of a team project focused on integration of NA assays with centrifugal microfluidics devices.

Michel Bergeron, MD, is professor and chairman of the Centre de Recherche en Infectiologie of Université Laval in Québec City, and the founder of Infectio Diagnostic Inc., which is now BD Diagnostic-GeneOhm.

Marc Madou, PhD, is a chancellor's professor in the mechanical and aerospace engineering department at UC Irvine, and a pioneer in the fields of centrifugal microfluidics and microfabrication.

The authors can be reached at jsiegris@uci.edu, regis.peytavi@crchul.ulaval, michel.g.bergeron@crchul.ulaval, and mmadou@uci.edu, respectively.