Colorimetric Micro-Paper-Based Analytical Devices: Simple or Complex Diagnostic Assays?

Brittany M. Rauzan Literature Seminar September 18, 2014

In response to the need for diagnostic assays for underdeveloped countries as defined by WHO as being "ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to end-users",[1] increasingly more research is directed towards paper-based analytical devices. In 2007, the Whitesides' lab first developed micro-paper-based analytical devices (μPADs). μPADs are light-weight, able to be patterned,[2] and can be produced at a cost of 0.001 cent per device.[3] While the devices were initially developed using photolithography,[4] the fabrication process was later simplified to use inkjet and wax printers.[1, 3, 5] The devices require a drop of blood from a finger prick and use colorimetric analysis, which eliminates the need for a laboratory and in theory for a trained technician.[1, 6] Since the device is paper-based it can be burned, which eliminates bio-hazardous waste.[7]

One critical application of colorimetric μPADs is performance under triage settings. For these demanding environments, μPADs for liver function filter whole blood, with the plasma serving as the medium to rewet the channels of the device. If a particular enzymatic marker (analyte) is present, the printed assay reacts, resulting in a color change within 30 min of initiation of the test. While a maximum of three analytes could be detected on the device and calibration curves were developed, the test focused on determining presence of analyte in a spiked sample.[7]

Further research on the liver test has been conducted on producing a device with a color gradient scale to determine analyte concentration. One earlier prototype device created focused on the detection of Erioglaucine in water with an eight color gradient scale based on average color intensity analysis using Adobe Photoshop. While the ability to correlate color and concentration was established, the ability to distinguish between eight categories may pose difficulty for an unskilled technician.[8]

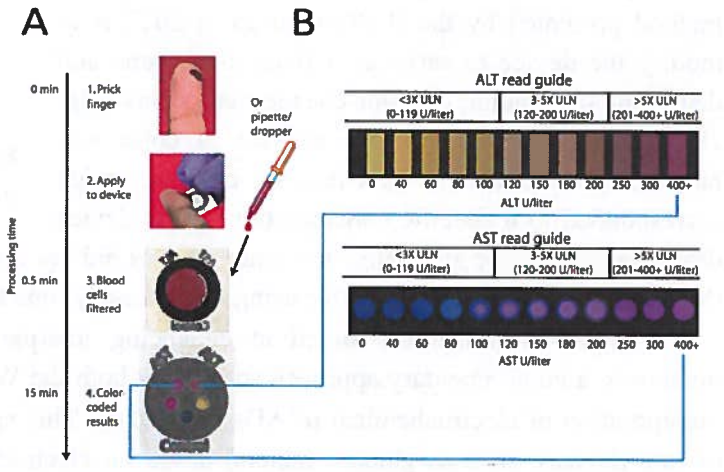

Figure 1. a) using device, b) color gradients and reading guide of AST and ALT[9]

In order to improve reading color gradients and increase the amount of attainable information, modified liver μPADs with a color gradient scale were maintained with a simplified interpretation classification scale (three categories: low, medium, high) (Fig. 1).[9] With further reduction to two categories, the number of undercalls was reduced, and the number of overcalls remained unchanged. While the device readability was improved, the level of detail was reduced, which limits its triage applications.[10]

An alternative is incorporation of telemedicine, which digitizes results to send to an expert for interpretation, as seen in μPADs for glucose and BSA detection with six concentration categories. The results are quantitatively analyzed with software to determine analyte concentration. While software interpretation improves accuracy in determination of concentration, there is the potential for error due to image quality originating from the type of camera used (digital vs. phone) and lighting conditions.[11]

As an alternative, a point-of-care transmittance colorimeter that can be used remotely has been developed. The device was able to perform single assay detection and has a control zone to account for lighting conditions. While the interpretation of results is improved with the device, the simplicity of the μPAD is reduced due to the need for an external device.[12]

The Chailapakul group in 2010 developed an alternative approach to colorimetric detection using three oxidative indicators for detection of analytes (Figure 2a), which made it easier to distinguish between different concentration gradients. When this system was compared to a one color gradient system, the multi-color device had a higher percentage of interpretation accuracy than single color gradient.[6]

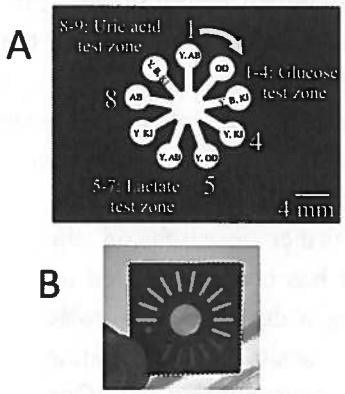

Figure 2. a) multi-color, b) spiral pattern[6, 13]

While using multiple colors does improve accuracy over one color gradient systems, an additional method presented by the Phillips group in 2012 is to modify the device to serve as a fixed assay time and determine the amount of color change that occurs (Fig. 2b). The quantification of the amount of color was based on the number of bars that are colored, which corresponded to a specific concentration. This device design is a prototype and unlike the other devices did not involve biological samples. However, this design serves as inspiration for using a fixed assay time to determine analyte concentration.[13]

While modifications aimed at enhancing interpretation of colorimetric μPADs are numerous, a complimentary approach studied by both the Whitesides and Chailapakul groups is incorporation of electrochemical μPADs (EμPADs). This approach shows promise since point-of-care devices, such as glucose meters) based on electrochemical readouts are already being used in clinical settings.[2] One approach within EμPADs is electrochemiluminescence, which was first reported in μPADs in 2011 by the Hogan group. Their device, which can be produced using an inkjet printer, uses $Ru(bpy)_3^{2+}$. The interpretation of the device requires a potential to

be applied, and still must be imaged using a camera phone and requires software analysis, but is not sensitive to lighting.[14] As a result, though the lack of uncertainty in results interpretation is reduced, the simplicity of the setup remains complex in its reliance on a reader for interpretation in comparison to the liver function colorimetric μPADs that could be interpreted without additional equipment.[7, 9]

Transitioning into EμPADs although promising is not absolutely necessary and rather should be viewed as a complimentary approach;[2] the colorimetric μPADs still offer a simplistic diagnostic assay. While EμPADs eliminate the errors due to environmental interferences with color reading,[2, 14] the modifications to coloremetrics as previously discussed illustrate that while the design is becoming more complex, the interpretation does not have to follow this trend and simplicity can be maintained.

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