Tear Analysis by Direct Nanoliter Specimen Collection Utilizing Lab-on-a-Chip Technology

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The use of tear fluid as a matrix for in vitro diagnostics has been largely constrained by the challenges of specimen collection and the technical complexity of nanoliter (nL) assays. A new class of lab-on-a-chip assays that combine direct nanoliter specimen collection and analysis into a single step are poised to change that paradigm - by eliminating pre-analytical errors from hypooosmolar reflex tearing and evaporation from sample manipulation.

Effective tear analysis overcomes two unique challenges not previously encountered in standard in vitro diagnostic testing:

1. Collecting a specimen from a very small source of tear fluid - often from a patient presenting with “dry eye” disease [1]. Tear samples must be collected in sufficient quantity to perform analysis without inducing reflex tearing, which would invalidate the specimen due to a dilution effect. The volume necessary to meet this requirement is 50 nanoliters or less.

2. The ability to analyze nanoliter volumes of tear, while avoiding pre-analytical errors due to evaporation during specimen handling, transfer or transport [2].

The combination of nanoliter sample collection and analysis has historically proven to be very difficult, thereby limiting the incorporation of in vitro diagnostics into the clinical practice of ocular medicine.

However, lab-on-a-chip technology capable of simultaneous nanoliter volume specimen collection and analysis has become available in the past decade and opens the door to in vitro diagnostics using tear samples. The first in a series of expected tear fluid tests has been developed utilizing this methodology and is “intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease, in conjunction with other methods of clinical evaluation” (The TearLab™ Osmolarity System - FDA k083184).

The TearLab™ Test Card is a lab-on-a-chip platform that enables tear fluid testing by the direct collection and analysis of nanoliter samples. The Test Card is a single-use, individually packaged, non-sterile, polycarbonate lab-on-a-chip containing (a) a sigmoidal nanofluidic channel to collect 50 nL of tear fluid sample by passive capillary action and (b) gold electrodes embedded in the polycarbonate card to enable measurement of the tear fluid sample within the nanofluidic channel.

The hallmark of the TearLab™ Osmolarity System is that it enables simultaneous collection and measurement of nanoliters of tear fluid. By combining the collection and analysis chambers onto a lab-on-a-chip, the TearLab™ test can be performed in less than 10 seconds, thereby simplifying the assay, eliminating sample transfer, sample preparation, and removing the risks of evaporative pre-analytical error.

The necessity to simultaneously collect and analyze nanoliter samples of fluid mandates that the specimen collection channel be held to the same exacting manufacturing tolerances required for analysis. This is a distinct technological attribute of the TearLab™ Test Card. Whereas microliter sampling technologies can use arbitrary low-precision devices, (i.e., inexpensive extruded glass capillary tubes or strips of lateral flow filter paper), nanoliter analysis dictates channel manufacturing tolerances of less than 3 microns (millions of a meter). Moreover, Test Cards must be manufactured in an ISO 13485 clean-room environment because even single molecule layers of contamination impact the accuracy of the sensor. The end result is a single-use device with a osmolarity coefficient of variation of less than 2%.

Similarly, tear collection places its own set of requirements on the design of the TearLab™ Test Card. Unlike traditional blood sampling techniques, the interface to the eye imposes safety considerations that require a blunt-ended plastic device – a geometry that would normally impede tear collection by capillary action. The tear collection interface on the TearLab™ Test Card uses a specially designed hydrophobic/ hydrophilic stack of materials to guide the inferior tear lake into the sample collection channel. Deemed “fluid focusing,” this method of tear collection is an innovation of the nanofluidic process.

Another consideration of tear testing is that the nanoliter fluid samples are not visible to the naked eye. The use of embedded electrodes within the lab-on-a-chip allows the TearLab™ to continuously monitor tear collection. Upon detecting 50 nL of fluid, the TearLab™ will signal the user through audio and visual feedback that sufficient fluid has been collected. Immediately upon collection, the TearLab™ initiates a series of measurements within the nanofluidic channel before evaporation corrupts the signal (which occurs approximately 10 seconds after collection). To maximize accuracy, a temperature-compensated, four-point impedance cell is embedded within every sample collection channel. Such innovations overcome the traditional obstacles of tear testing, and ensure that sample collection, which is just as vital to an accurate, precise test as the analytical method, is an integral component of the assay.

In summary, “tear analysis by direct capillary nanoliter specimen collection” necessitates a technology platform that meets the following requirements:

- **Sample volumes** of 50 nanoliters or less
- **Safe Collection** – treated plastic microchannel to enable passive capillary sampling without the risks of glass shards or abrasion
- **Mitigation of Evaporative Error** – sample analysis completed within 10 seconds of collection
- **No Sample Transfer** – direct analysis within the capillary collection channel
- **Collection Monitoring** – real-time feedback to alert the user as to when a sample invisible to the naked eye has been collected
- **Analysis** – laboratory quality performance in a nanofluidic environment at the point-of-care

Abbreviations: DED, dry eye disease;
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References


Fig. 1. (A) The TearLab™ Osmolarity system. The TearLab Osmolarity system is intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease, in conjunction with other methods of clinical evaluation. It is comprised of the reader, pen and a disposable test card. (B) In clinical practice, tears are collected directly from the inferior lateral tear meniscus. (C) The single-use, disposable polycarbonate microchip (approximately 25mm x 12mm x 1mm) contains a sigmoidal microchannel (75 µm x 300 µm x 5mm) at the tip. The channel collects 50 nanoliters (nL) of tear fluid directly from the inferior meniscus of the ocular surface by passive capillary action. Gold electrodes embedded in the polycarbonate card enable measurement of the electrical impedance of the tear fluid sample in the channel.