Introduction

Gliomas are the most common primary malignant brain tumors in adults. Recurrent isocitrate dehydrogenase (IDH) gene mutations are found in up to 80% of low grade gliomas and 20% of secondary glioblastomas. Cell-free DNA is released into biofluids from dying cellular processes and has been used to detect mutations in plasma from cancer patients but has been a challenge in gliomas. Blood brain barrier (BBB) is a dynamic but complex barrier that exosomes but not larger particles can cross.

On the other hand, exosomes and other extracellular vesicles are actively released from living processes into plasma, and combining these two sources of nucleic acids increases sensitivity. The goal of this study was to develop a droplet digital PCR-based assay to detect the IDH1 R132H mutation from plasma samples of glioma patients using cDNA and exosomal nucleic acids (exoNA) combined as input material (Figure 1).

![Image](image1.png)

Figure 1. Origin of nucleic acids in biofluids. Apoptotic or necrotic cells may release cell-free DNA (cDNA) in apoptotic vesicles (A) or as circulating nucleosomes (A'). Exosomes are actively released by living cells (B) carrying nucleic acids into circulation (exona).

Material and Methods

We developed a plasma-based assay that combines exoNA and cell free DNA to detect single copies of R132H mutation with ~2 μL of input material using ddPCR (Figure 2).

The clinical performance was assessed on an interim cohort of 16 positive and 8 negative R132H clinical samples confirmed by matched tissue data (Table 1). Three additional healthy samples were also processed as negative controls.

![Image](image2.png)

Figure 2. Assay workflow (A) Exosome-derived nucleic acids and cDNA isolation from plasma. (B) Reverse transcription step that includes Qbeta as a control of inhibition. (C) Pre-amplification step. (D) Quality Control step by qPCR to ensure absence of enzymatic inhibitors. (E) Droplet Digital PCR that detects R132H within IDH1.

Results

We detected R132H in the patient samples analyzed with 56% sensitivity and 91% specificity (Figure 3, Table 2). This result is similar to the sensitivity we previously published using exosomes from cerebrospinal fluid, however plasma is a much more accessible biofluid for diagnostics.

![Image](image3.png)

Figure 3. Example of droplet digital PCR results from one of the R132H positive patient samples by tissue. 2-Dimensional plot that shows a clear separation between the different clusters of droplets (positive for either channel or negative).

Table 2. Confusion matrix that summarizes the clinical samples tested.

<table>
<thead>
<tr>
<th>Plasma results using</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>cDNA and exosomal NA</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
</tr>
<tr>
<td>result*</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10</td>
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<td></td>
<td>11</td>
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*All four healthy samples have been included as tissue negative.

Conclusions

- First novel platform able to capture both exoNA and cDNA from plasma from patients with low grade gliomas to detect R132H.
- Comparable sensitivity to a previous study that used Cerebrospinal Fluid, with the advantage of being less invasive.
- This is a promising result for brain cancer since this is a particularly challenging disease for peripheral biomarkers.
  - When the same exoNA isolation platform was utilized across 307 patients in different peripheral cancers this platform achieved a sensitivity ranging from 90-98% and specificity of 92%.

References