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# Au@Pt Nanoparticle Encapsulated Target-Responsive Hydrogel with Volumetric Bar-Chart Chip Readout for Quantitative Point-of-Care Testing\*\*

Zhi Zhu, Zhichao Guan, Shasha Jia, Zhichao Lei, Shuichao Lin, Huimin Zhang, Yanli Ma, Zhong-Qun Tian, and Chaoyong James Yang\*

**Abstract:** Point-of-care testing (POCT) with the advantages of speed, simplicity, portability, and low cost is critical for the measurement of analytes in a variety of environments where access to laboratory infrastructure is lacking. While qualitative POCTs are widely available, quantitative POCTs present significant challenges. Here we describe a novel method that integrates an Au core/Pt shell nanoparticle (Au@PtNP) encapsulated target-responsive hydrogel with a volumetric bar-chart chip (V-Chip) for quantitative POCT. Upon target introduction, the hydrogel immediately dissolves and releases Au@PtNPs, which can efficiently catalyze the decomposition of  $\text{H}_2\text{O}_2$  to generate a large volume of  $\text{O}_2$  to move of an ink bar in the V-Chip. The concentration of the target introduced can be visually quantified by reading the traveling distance of the ink bar. This method has the potential to be used for portable and quantitative detection of a wide range of targets without any external instrument.

The development of point-of-care testing (POCT) is of great importance to improve healthcare, ensure environmental safety, and guarantee food quality, especially in a variety of environments that lack access to laboratory infrastructure, such as in less-industrialized countries, in emergency situations, or in home healthcare settings.<sup>[1]</sup> The basic criteria for POCT require it to be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to the end user, which corresponds to the acronym “ASSURED” by the World Health Organization (WHO).<sup>[2]</sup> While qualitative POCTs are widely available as dipsticks and lateral-flow systems, quantitative POCTs present significant challenges that cannot be easily achieved in an inexpensive

and convenient manner.<sup>[3]</sup> However, quantitative assays are important when a “yes” or “no” answer is insufficient. For example, in many cases the presence of cancer biomarker protein in a patient’s blood sample does not suggest the occurrence of cancer unless its concentration is above a diagnostic threshold.

In our daily life, some classical physical principles are widely applied in visually quantitative devices, such as thermometers, barometers, and hygrometers. Such devices are ideal models for POCT, as they provide simple, portable, sensitive, and quantitative detection without any external instrument or power source. However, these devices can detect only a few physical parameters, such as temperature, pressure, and humidity. The combination of these physical principles with some chemical or biological recognition elements has great potential to extend such simple, yet quantitative, devices for the detection of various types of targets.


Very recently, the Qin research group designed an elegant multiplexed volumetric bar-chart chip (V-Chip) for quantitative POC diagnostics.<sup>[4]</sup> Based on SlipChip technology,<sup>[5]</sup> the V-Chip uses ink bar charts to indicate the pressure change in a channel as a result of  $\text{O}_2$  generation from the reaction of catalase with  $\text{H}_2\text{O}_2$  for target quantification. The V-Chip method cleverly translates molecular recognition events into an easily measurable physical parameter, that is, volume of oxygen gas. However, there are two important limitations that need to be resolved before the design can be widely adopted in the field of POCT. First, the requirement of conjugating catalase and an antibody is time consuming and may affect the structure and function of proteins to a certain extent. Second, the brief lifespan of catalase in its  $\text{H}_2\text{O}_2$  substrate solution significantly compromises the sensitivity of the assays.<sup>[4b,6]</sup> Thus, it is desirable to develop new signal transduction strategies and new catalysts that allow simple sample processing without protein modification, while still providing efficient yet robust conversion of the target recognition event into visually measurable parameters. Herein, we present a simple and general quantitative POCT method by integrating a target-responsive hydrogel for target recognition,<sup>[7]</sup> Au core/Pt shell nanoparticles (Au@PtNPs) for robust yet highly efficient signal transduction and amplification, and a volumetric bar-chart chip for visual quantitative readout.

The basic design of the target-responsive hydrogel was adapted from our previous studies based on an enzyme-encapsulated aptamer-cross-linked hydrogel.<sup>[7b-d]</sup> Although we have achieved quantitative detection with a personal

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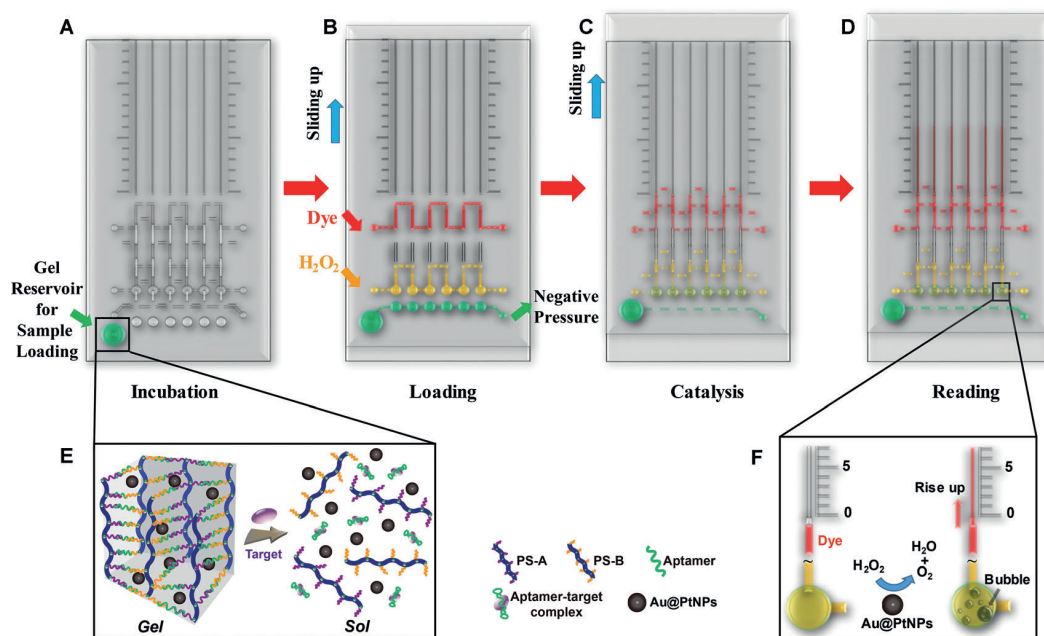
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glucose meter, it still requires an external electronic detection device, and the integration of a hydrogel with the glucose meter cannot be easily achieved. Therefore, to realize a “detector-less” integrated quantitative POCT, we propose the concept of a target-responsive hydrogel integrated with a volumetric bar-chart chip (HV-Chip) readout for the sensitive, selective, and quantitative POCT by the naked eye. The working principle of the HV-Chip is shown schematically in Figure 1. To prepare the Au@PtNPs-encapsulated target-responsive hydrogel, DNA strands A and B are grafted onto linear polyacrylamide polymers to form polymer strands A and B (PS-A and PS-B), respectively. The hybridization of the two strands A and B with a linker aptamer yields a three-stranded complex to cross-link PS-A and PS-B into a hydrogel. Au@PtNPs, which can catalyze the decomposition of  $\text{H}_2\text{O}_2$  to generate  $\text{O}_2$ , are preloaded inside the hydrogel during its formation. The prepared gel is kept in the gel reservoir of the V-Chip (Figure 1A). When target molecules are added to the gel reservoir, the aptamer-target complex forms, which leads to dissociation of the hydrogel into a sol and release of the encapsulated Au@PtNPs into the supernatant solution (Figure 1E). The top layer of the V-Chip is then slid up to the loading position by hand, thereby forming three connected channels in the horizontal direction (Figure 1B). A negative pressure is applied with a pipette to the outlet of the bottom connected channel (green), to draw the supernatant into the channel through the drilled hole at the bottom of the gel reservoir. Subsequently, the top connected channel (red) of the V-Chip is loaded with red ink and the middle connected channel (yellow) with  $\text{H}_2\text{O}_2$ . After loading the supernatant and reagents, the V-Chip is slid upward further to separate the

horizontal fluidic paths and form six independent parallel connected channels in the vertical direction (Figure 1C), where the supernatant containing the Au@PtNPs is brought into contact with  $\text{H}_2\text{O}_2$  in each individual channel. Instantly,  $\text{O}_2$  is generated and the ink is pushed up into the top thinner channel (Figure 1D,F). The distance that each ink bar moves within a specified time is independently correlated with the concentration of the Au@PtNPs catalyst, which are generated from the hydrogel in proportion to the target concentration. Thus, the target can be visually and quantitatively detected without the need for an external electronic device and power source. More importantly, Since one target can trigger the release of many Au@PtNPs from the hydrogel and one Au@PtNP can catalyze many rounds of reactions to generate a large volume of  $\text{O}_2$ , the sensitivity of the method can be greatly enhanced, while the specificity is guaranteed by the high selectivity of the aptamer.<sup>[8]</sup> Therefore, the proposed HV-Chip method can provide quantitative, portable, sensitive, selective, and visual POCT.

The V-Chip device with six channels in parallel for single sample testing is fabricated using two  $63 \times 63 \times 1.5$  mm glass slides. Patterns are first drawn with AutoCAD software (see Figure S1 in the Supporting Information) and fabricated onto the glass surfaces by standard photolithography and glass etching methods (see the Supporting Information). Once assembled and aligned in a specific configuration in the presence of fluorinated oil, a fluidic path is formed. The supernatant or reagents are preloaded through drilled holes using a pipette. Then, the top plate is moved relative to the bottom plate to enable mixing and reaction of the samples or reagents. In contrast to the reported patterns of multiple-channel V-Chip with relatively oblique sliding,<sup>[6]</sup> the designed



**Figure 1.** The working principle of the Au@Pt nanoparticle encapsulated target-responsive hydrogel with a volumetric bar-chart chip readout for visual quantitative detection.

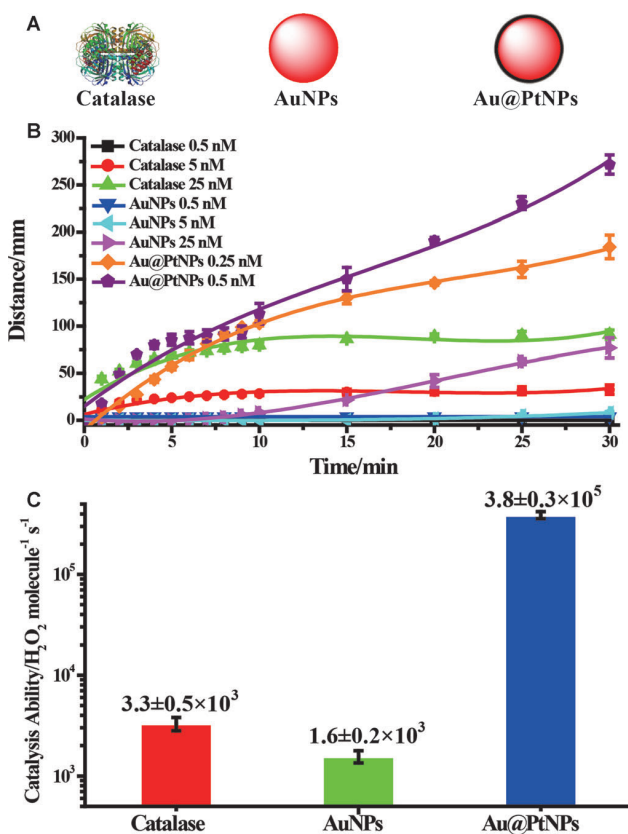
pattern allows to alternate between the loading and reading position by vertical sliding, thereby resulting in easy operation and calibration during the experiment.

To ensure the high sensitivity of the assay, we first needed to find an efficient and stable catalyst to catalyze the decomposition of  $\text{H}_2\text{O}_2$  to generate  $\text{O}_2$ . A serpentine single-channel V-Chip (see Figure S2 in the Supporting Information) was fabricated for this investigation, which allowed long-time reaction and observation. For each catalyst, the reaction was monitored by taking pictures every minute in the first 10 min and every 5 min from 10 to 30 min. The distance travelled by the ink was measured individually, and the time-dependence of ink advancement was plotted (Figure 2B). The enzyme catalase, as one of the most efficient enzymes found in nature, was first tested. As shown in Figure 2B, a catalase concentration of 0.5 nM could not generate sufficient  $\text{O}_2$  to push the ink, but the distance moved by the ink bar increased as the catalase concentration was increased. However, the catalase failed to catalyze the  $\text{O}_2$ -generation reaction after 10 min, because of destruction of the catalase by  $\text{H}_2\text{O}_2$ .<sup>[6]</sup> Alternatively, nanoparticles have recently been reported as catalase mimics with good stability and high catalytic efficiency.<sup>[9]</sup> Therefore, we synthesized 13 nm AuNPs,<sup>[10]</sup> but the catalytic ability of the AuNPs was lower than that of

catalase. As a consequence of the excellent catalytic ability of Pt for  $\text{H}_2\text{O}_2$  decomposition,<sup>[11]</sup> a thin Pt layer was deposited on the AuNP surface to form Au core/Pt shell nanoparticles (Au@PtNPs).<sup>[12]</sup> The Pt coating led to a dramatic increase in the catalytic efficiency. At a concentration as low as 0.25 nM, the Au@PtNPs pushed the ink bar 180 mm in 30 min, compared to 100 mm by 25 nM catalase and 80 mm by 25 nM AuNPs. Moreover, the Au@PtNPs can steadily catalyze the generation of  $\text{O}_2$  over a long period of time, thus indicating the excellent stability of the Au@PtNPs (typical images of the Au@PtNP-catalyzed reaction over time, and the images of all the catalysts at 25 min and 30 min are shown in Figure S4 in the Supporting Information). By measuring the channel dimensions (see Figure S5 in the Supporting Information) and calculating the amount of  $\text{O}_2$  generated, the apparent turnover number in 30 min was evaluated as the number of  $\text{H}_2\text{O}_2$  molecules decomposed per catalyst molecule per second (Figure 2C). The catalytic efficiency of the Au@PtNPs was found to be more than two orders of magnitude higher than that of catalase, which is one of the most efficient enzymes found in nature. Therefore, the Au@PtNPs with their high catalytic efficiency and good stability were chosen as the catalyst for subsequent experiments.

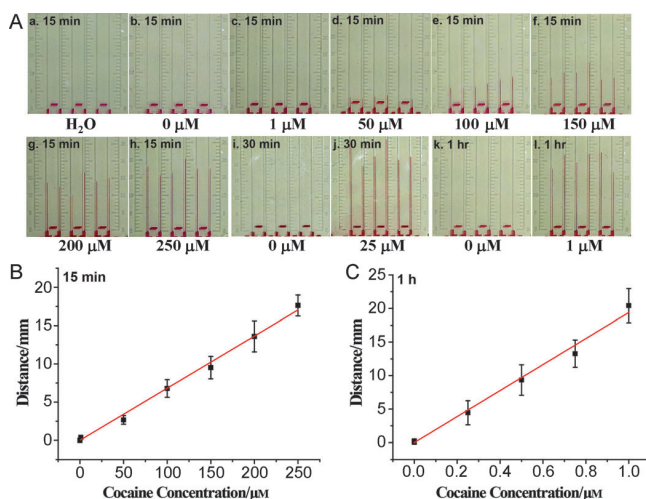
To investigate the feasibility of the HV-Chip system, an Au@PtNP-encapsulated cocaine-responsive hydrogel was used. The simple, fast, and quantitative detection of small doses of cocaine, a highly addictive drug, is essential to prevent its abuse. The hydrogel was prepared from 4% acrylamide and the cross-linking DNA, whose final concentrations were optimized to be 110  $\mu\text{M}$  PS-A, 110  $\mu\text{M}$  PS-B, and 66  $\mu\text{M}$  aptamer. The final concentration of Au@PtNPs in the hydrogel was determined to be around 4.6 nM, which is the highest concentration of Au@PtNPs we could obtain. At that concentration, there was no observable leakage of Au@PtNPs from the hydrogel. We also demonstrated that without catalyst, there was no auto-decomposition of  $\text{H}_2\text{O}_2$  on the chip (Figure 3Aa). Afterwards, the HV-Chip method was applied to quantify the concentration of the cocaine target. Different concentrations of cocaine solution were prepared. In the initial proof of concept experiment, reactions were carried out in Eppendorf tubes instead of a gel reservoir with 10  $\mu\text{L}$  of the hydrogel and 50  $\mu\text{L}$  of a solution phase containing cocaine. The tubes were shaken gently at 150 rpm and 25  $^\circ\text{C}$  for 1 h. Then, 5  $\mu\text{L}$  of the supernatant was transferred for V-Chip readout.

In the V-Chip, the dye solution,  $\text{H}_2\text{O}_2$ , and sample solution were added to the appropriate locations at the loading position. Then, the top layer was slid up to the reading position for mixing, the  $\text{O}_2$ -generation reaction, and reading. Cocaine concentrations ranging from 0 to 250  $\mu\text{M}$  were tested with six repeated measurements each in parallel. Figure 3A–h shows the target-responsive bar chart results after 15 min. The distance moved by the ink bar gradually increased as the target concentration increased. Figure 3B shows that the measured distance was linearly proportional to the concentration of cocaine, thus establishing the quantitative detection capability of the HV-Chip method. In addition to its outstanding catalytic ability, another advantage of the Au@PtNPs as a catalyst is their excellent stability. If sufficient



**Figure 2.** Comparison of the catalytic ability of catalase, AuNPs, and Au@PtNPs. A) Schematic representation of the three catalysts. B) Time-dependent ink advancement with different types of catalysts. C) The catalytic ability evaluated as the number of  $\text{H}_2\text{O}_2$  molecules decomposed per catalyst molecule per second.





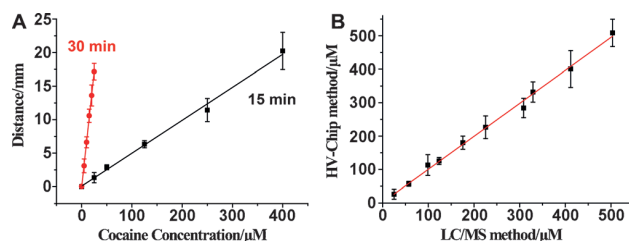
**Figure 3.** Performance of the HV-Chip method for the detection of cocaine. A) Images showing ink advancement for the detection of cocaine in 15 min (a–h), 30 min (i,j), and 1 h (k,l). Linear standard curves were obtained from 0 to 250  $\mu\text{M}$  in 15 min (C) and 0 to 1  $\mu\text{M}$  in 1 h (D).

$\text{H}_2\text{O}_2$  is provided, the catalytic reaction can continue for a long time. Better sensitivity was achieved by simply prolonging the reaction time from 15 min to 30 min (Figure 3A,i,j; see also Figure S6 in the Supporting Information) or 1 h (Figure 3A,k,l; see also Figure S7 in the Supporting Information) with saturated  $\text{H}_2\text{O}_2$ . For example, the chip with a cocaine concentration of 1  $\mu\text{M}$ , which showed little response in 15 min, can induce significant advancement of the ink bar to achieve a much lower limit of detection (LOD). As shown in Figure 3C, the HV-Chip method can detect cocaine lower than 1  $\mu\text{M}$  in a linear fashion with a LOD of 0.06  $\mu\text{M}$  (calculated from  $3\sigma_b/\text{slope}$ , where  $\sigma_b$  is the standard deviation of 6 blank samples), which is about two orders of magnitude lower than that of our previous PGM-hydrogel method.<sup>[7d]</sup> Furthermore, the selectivity of the method was demonstrated (see Figure S8 in the Supporting Information) by showing that the travel distance in response to 250  $\mu\text{M}$  cocaine is 40 times higher than that of 2.5 mM cocaine metabolites. These results clearly demonstrated that the HV-Chip method can allow quantitative detection with the naked eye with excellent sensitivity and selectivity.

After demonstrating the feasibility of the HV-Chip method, we used the V-chip with an integrated gel reservoir (see Figure S9 in the Supporting Information) to evaluate the capability of the method for the detection of cocaine in urine. The DNA hydrogel was preloaded in the gel reservoir. After adding sample into the reservoir and incubation for 1 h, dissociation of the gel took place. The supernatant and reagents were then loaded according to the aforementioned procedure. The distances advanced by the ink under different cocaine concentrations after 15 min (see Figure S10A in the Supporting Information) and 30 min (see Figure S10B) were recorded. Both of them exhibited a good linear relationship between the cocaine concentrations and the measured distances (Figure 4A). A higher sensitivity was obtained when the catalysis time was increased from 15 min to

with a LOD of 0.33  $\mu\text{M}$ , lower than the initial test cutoff concentration (0.5  $\mu\text{M}$ ) set by the Department of Health and Human Services in the US. Only small variations were observed in assays performed on several separate devices, which suggests the assay is reproducible. To confirm the accuracy of the HV-Chip method, the quantitation results obtained by the HV-Chip method for 10 different concentrations of cocaine in urine were compared with the results acquired by a standard LC/MS method. A strong positive correlation between these two methods was found (Figure 4B). These results established that the accuracy and reliability of our method is as good as that of the standard LC/MS method for real sample analysis.

Since DNA hybridization is the only requirement of the aptamer–hydrogel design, our HV-Chip method should have the versatility to detect other targets, simply by changing the aptamer sequence in the target-responsive hydrogel. To verify such a generality, we designed an ATP HV-Chip method based on the same principle by using an ATP-responsive hydrogel. This ATP HV-Chip method allowed quantitative analysis of ATP with a linear range from 0 to 1 mM (see Figure S11 in the Supporting Informa-



**Figure 4.** Detection of cocaine in urine. A) Linear standard curves were obtained from 0 to 500  $\mu\text{M}$  in 15 min and 0 to 25  $\mu\text{M}$  in 30 min. B) Comparison of the HV-Chip method with the standard LC/MS method based on 10 samples in urine.

tion). Therefore, this strategy is a generic approach that can be modified with different aptamer sequences for the portable and quantitative detection of a wide range of targets without any external electronic devices.

In summary, a simple and general quantitative POCT method was developed by integrating target-responsive hydrogels for target recognition, Au@PtNPs for robust yet highly efficient signal transduction and amplification, and a volumetric bar-chart chip for a visual quantitative readout. The method allows the inexpensive, rapid, portable, and quantitative visual detection of a wide range of targets without any external electronic devices, thereby offering several advantages. First of all, the method is designed to meet the ASSURED standard<sup>[2]</sup> with the ability for quantification. Second, the high catalytic efficiency and good stability of the Au@PtNPs trapped inside the hydrogel provide a quantitative and highly sensitive relationship between the target concentration and V-Chip readout. Moreover, as a variety of aptamers against a broad range of targets are either available or can be obtained through SELEX,<sup>[13]</sup> the method developed here can be used as a powerful tool for the

detection of other targets simply by inclusion of specific aptamer sequences into the hydrogel. The HV-Chip method with the advantages of high sensitivity, selectivity, versatility, robustness, low cost, power-free, and portability has the great potential to be used for quantitative POCT by local communities in developing regions to improve healthcare, environmental safety, food quality, etc.

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**Keywords:** aptamers · hydrogels · microfluidics · point-of-care testing

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## Communications

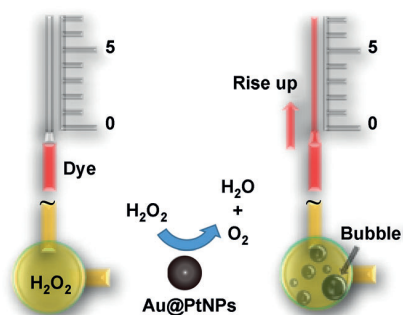


### Point-of-Care Testing

Z. Zhu, Z. Guan, S. Jia, Z. Lei, S. Lin,  
H. Zhang, Y. Ma, Z. Tian,  
C. Yang\*



Au@Pt Nanoparticle Encapsulated  
Target-Responsive Hydrogel with  
Volumetric Bar-Chart Chip Readout for  
Quantitative Point-of-Care Testing



**Seeing is believing:** A simple and general quantitative method has been developed by integration of target-responsive hydrogels, Au core/Pt shell nanoparticles to catalyze the decomposition of  $\text{H}_2\text{O}_2$  to  $\text{O}_2$ , and a volumetric bar-chart chip for a visual quantitative readout. A wide range of targets can be detected without any external electronic devices, as demonstrated for cocaine, with a detection limit of  $0.33 \mu\text{M}$  in urine.