



# A 96-well wax printed Prussian Blue paper for the visual determination of cholinesterase activity in human serum

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## ABSTRACT

In the last decades, there is a growing search for analytical strategies to ensure clinical analysis without the need of laboratory set-up and skilled personnel. Indeed, user-friendly and low-cost devices are highly valued in the era of sustainability for their capability to be applied in low-resource contexts, such as developing countries. To address this issue, herein we report a 96-well paper-based and laboratory setup-free optical platform for the detection of butyrylcholinesterase enzyme (BChE) activity in human serum. We used chromatographic paper to realize a novel analytical tool exploiting its porous structure for reagentless synthesis of Prussian Blue Nanoparticles (the sensing element), as well to load all the reagents required for the measurement. The principle of BChE activity detection relies on the reaction between the enzymatic product thiocholine and Prussian Blue, giving the Prussian White with subsequently Prussian Blue's fading, detected by a common office scanner supported by ImageJ software. Using this novel paper-based optical platform, BChE activity was linearly detected in the 2–15 U/mL range with a detection limit down to 0.8 U/mL. The accuracy was successfully demonstrated by recovery study with spiked serum and by comparing the data with the gold standard method.

## 1. Introduction

Demand for smart analytical tools increases year-by-year to fill in the gap in diagnostic sector. For instance, the developing world needs of cost-effective, robust, accurate, and easy to use devices to take advantage of the treatments available today for the proper disease management (Urdea et al., 2006). Indeed, the absence of effective and in-situ monitoring in remote areas is amenable as the first cause of death (Mabey et al., 2004). To address this problem, several research programmes e.g. EU, NIH, Bill and Melinda Gates, Wellcome Trust, boosted the development of low-cost and rapid solutions performed at low-infrastructure sites. Several portable analytical tools have been designed for the detection of biomarkers such as nucleic acids, enzymes, small molecules, etc. (Drain et al., 2014; Labib et al., 2016; Roda et al., 2018), beyond the well-established blood glucose strip (Turner, 2013; Witkowska Nery et al., 2016).

Starting from 2008, the world of point-of-care (POC) devices has lighted up a rising star: paper (Martinez et al., 2009; Vashist et al., 2015; Yang et al., 2016; Arduini et al., 2017; Salentijn et al., 2018; Cinti et al., 2017). Even if paper has been employed earlier in analytical tools

(i.e. litmus paper, pregnancy test), it is now attracting researchers working in the field of (bio)sensors. The increasing development of paper-based (bio)sensors is ascribed to valuable features of paper such as abundance, low-cost, 3D porous structure, sustainability, and incinerability. Overall, the advantageous employment of paper in realizing smart colorimetric and electrochemical devices is widely reported in literature: a “pop-up” device for analysis of beta-hydroxybutyrate (Wang et al., 2016); an “in writing” paper for blood typing, (Li et al., 2012); a multiple “3D origami” platform for pesticide detection (Arduini et al., 2019), represent just few examples of successfully paper-based devices. Recently, our group highlighted a brand-new feature of paper: the structure and composition of low-grade filter paper has been exploited for the eco-designed synthesis of Prussian Blue nanoparticles (PBNPs), just starting from precursors, without employing the use of reactants, i.e. reducing agents, for electrochemical detection of glucose by monitoring glucose oxidase by-product (i.e. hydrogen peroxide) (Cinti et al., 2018a). However, even if electroanalysis is characterized by several advantages such blindness towards colored/turbid matrices, sensitivity, and robustness, there are some experimental aspects that make colorimetric methods closer to end-users. For

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instance, color appearance or fading is easily followed by using human detectors, i.e. eyes. Furthermore, the simultaneous multiplex analyses read-out is easily available for optical measurement in contrast with electrochemical analyses for which the read-out is usually available only for sequential analyses (Cinti et al., 2018b). In this work, for the first time, we merge the reactor-like feature of paper with a standard 96-well configuration (compatible with plate readers, i.e. ELISA and multichannel pipettes) for the visual measurement of butyrylcholinesterase (BChE) activity in serum samples. The detection of BChE in serum is a relevant clinical analysis, because measurement of cholinesterase activity is a sensitive indicator of the liver capacity. In the absence of genetic causes or known inhibitors (organophosphorus compounds), any decrease in BChE activity reflects impaired synthesis of the enzyme by the liver. Furthermore, the determination of serum cholinesterase levels may be helpful in the pharmacological study of Alzheimer's disease (Kumada et al., 2011; Prado et al., 2014; Li et al., 2017). Moreover, BChE in blood has a crucial importance in anaesthetist practices for its role in the metabolism of anaesthetic related drugs such as suxamethonium (Davis et al., 1997), thus BChE measurement is often required before any surgery. Within the last five years, outstanding examples have been reported to detect the activity of cholinesterase enzymes in biological fluids e.g. serum and blood. In 2015, Lu and Xia detected the activity of cholinesterase in human blood by evaluating the change in surface plasmon resonance of gold nanorods as consequence of enzyme-modulated self-assembly. However, this approach is strictly dependent on the instrumentation. In 2016, paper has been adopted as the manufacturing substrate for the fluorescent visualization of cholinesterase activity in diluted serum samples (Chang et al., 2016). However, the paper-based substrate is only used to perform the visualization. This method is strictly linked to the use of several reagents and the detection limit of 2.5 U/mL is still high (perhaps due to the background fluorescence of paper). Recently, filter paper has been also adopted to electrochemically detect BChE activity in serum samples. Despite the simplicity associated with this approach, the measurement requires a potentiostat for BChE activity quantification (Scordo et al., 2018). In summary, all these approaches highlight several limitations, i.e. ad-hoc detector, external reagents needed, multi-steps, single quantification.

In this work, a 96-well layout was obtained using customized wax-printed Whatman No.1 paper. This configuration allows for some successful achievements: i) each well represents an independent platform, and this means the possibility to perform 96 simultaneous measurements; ii) the porosity of paper allows for loading all the required reagents; iii) the paper-based synthesized PBNPs behave as the display: the enzymatic by-product is able to oxidize PB that turns in its reduced colorless form (Prussian White, PW); iv) the unique PBNPs-chromatographic paper configuration allows for measuring serum samples: the 3D porous structure of paper coupled to the intense PB coloring offers the capability to filter gross impurities, going beyond some issues associated with the color of matrices. With the aid of cheap material (i.e. paper), affordable manufacturing methods (i.e. wax printing), and eco-reactor (i.e. PBNPs are synthesized autonomously within paper avoiding reducing agents), the activity of BChE is monitored without any addition of reagents (i.e. all chemicals are pre-loaded within the paper and only serum sample is needed for the measurement), lab-independently (i.e. mobile phone or scanner might be used), and without managing hazardous wastes (i.e. after its use, paper can be easily burned). It represents an example to how a known material as paper can be easily customized to provide a laboratory-independent tool to be used for multi-analytical purposes.

## 2. Materials and methods

### 2.1. Reagents and instruments

Butyrylthiocholine chloride, butyrylcholinesterase (BChE, from

equine serum), potassium ferricyanide ( $K_3Fe(CN)_6$ ), iron chloride ( $FeCl_3$ ), urea, glucose, uric acid, serotonin hydrochloride, L-cysteine (L-Cys), L-glutathione reduced (GSH), glucose oxidase (GOx) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were used without further purification. Serum samples from volunteers were provided by University Hospital of Rome Tor Vergata. Optical measurements were carried by scanning the 96-well paper-based platform with a common office scanner (Canon PIXMA, MP450) with an optical resolution of  $1200 \times 2400$  dpi, and the intensity of color was evaluated with the ImageJ software, an open source image-processing program. For BChE activity measurement by using reference method, MULTIGENT Cholinesterase method was employed by means of ARCHITECT System (Abbott) for clinical analyses located at University Hospital of Rome Tor Vergata.

### 2.2. Production of the paper-based multi platform

The paper-based platform was designed by taking the 96-well ELISA plate as the reference. Firstly, the pattern was designed with a drawing software (Adobe Illustrator) and printed onto chromatographic paper (Whatman No.1) by using an office wax printer (ColorQube 8580, Xerox, USA). In order to create 96 hydrophilic wells, isolated by hydrophobic edges, the waxed platform was cured in an oven at  $100^\circ C$  for 2 min. Successively, PBNPs were synthesized within each well by adding a mixture containing the precursors, potassium ferricyanide and iron chloride prepared in distilled water. In accordance with a procedure for synthesizing PBNPs previously reported by our group (Cinti et al., 2018a), 90 min in an oven at  $70^\circ C$  were selected to homogeneously cover the paper-based structure with PBNPs (Fig. 1). The mean size of the synthesized PBNPs was evaluated equal to  $25 \pm 4$  nm (Cinti et al., 2018a).

### 2.3. Visual evaluation of the BChE activity

The visualization of the activity of BChE is based on the fading of PB (Fig. 2). As reported in literature for the first time by our group, PB is known to electrocatalyze the oxidation of thiols e.g. thiocholine (Ricci et al., 2004). In this work, we exploited the capability of thiocholine (BChE enzymatic by-product) to reduce PB to Prussian White (PW) for the measurement of BChE activity. To carry out the visualization of BChE activity, each well of the paper-based platform was pre-loaded with  $5 \mu L$  of  $200 \mu M$  butyrylthiocholine previously dissolved in phosphate buffer (pH 7.4). When the sample containing BChE is added to the wells, the substrate (butyrylthiocholine) is enzymatically converted into the by-products butyric acid and thiocholine: the latter is capable

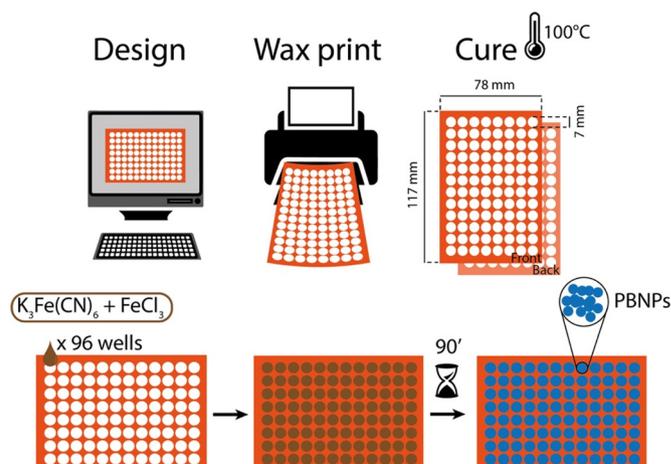


Fig. 1. Procedure for obtaining the 96-well paper-based multi-platform containing PBNPs.

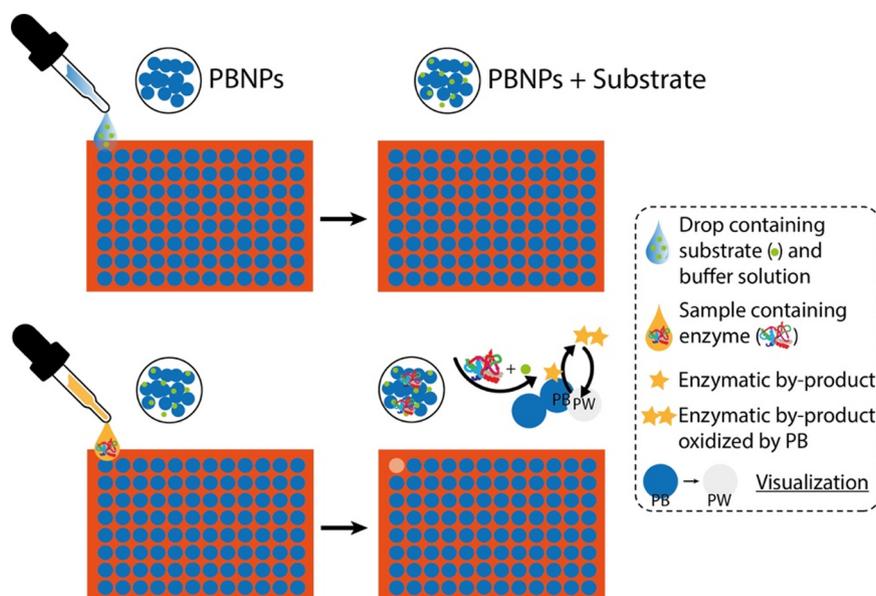


Fig. 2. Schematic representation of BChE activity visualization, including the enzymatic reaction and the PB fading.

to reduce PB to PW with the subsequent fading of blue color. The decrease of the blue color, due to conversion of PB to PW, is proportional to the activity of the enzyme, thus it was used for BChE activity visualization/quantification. The quantitative data were obtained by calculating the difference in mean color intensity of each well ( $\Delta I$ , i.e.  $I$  (standard solution or sample) –  $I$  (control, i.e. 0.1 M phosphate buffer solution, pH 7.4). Experimentally, the paper-based platform is conceived as reagent-free tool, being each of the 96 wells loaded with all the necessary reagents, i.e. the enzymatic substrate (butyrylthiocholine), and phosphate buffer to obtain the optimal value of ionic strength/pH for the highest activity of BChE. To perform the measurement, the end-user will be asked to drop few microliters of sample (containing BChE) and to evaluate the fading.

### 3. Results and discussion

#### 3.1. Optimization of the PBNPs-based platform

With the aim of producing a sensitive and reproducible platform, the first optimization was focused on the choice of the volume of the precursor mixture to be added to the paper-based wells, taking into consideration both the homogeneity of the final PBNPs and the sensitivity towards thiocholine (the enzymatic by-product that allows to evaluate the BChE activity), Fig. 3A. The study of the volume of precursors is a crucial investigation to avoid the coffee ring effect, i.e. liquid diffuses towards the edges of the hydrophilic area accumulating there and limiting the homogeneity of the final platform (through this effect, particles can be even discriminated by size) (Yunker et al., 2011; Wong et al., 2011).

As shown in Fig. 3A, the volume of precursor mixture was investigated in the range comprised between 3 and 20  $\mu\text{L}$ . The volume of 12.5  $\mu\text{L}$  was chosen for successive works due to homogeneous synthesis of PBNPs and the suitable sensitivity for both low and high concentrations of thiocholine. A lower volume was not selected due to a poor coverage of the well, while the use of higher volume led to very dark blue color that resulted not enough sensitive towards the analyte. Along with the optimization of the volume to be used for the PBNPs synthesis, the volume of sample to be added for the detection was evaluated. In this case, a volume of 5  $\mu\text{L}$  was chosen as the optimal compromise between the sensitivity and the amount of the sample. In this case, a lower volume is not sufficient to cover the entire well (thus reacting with all PBNPs), while a higher volume of sample highlights

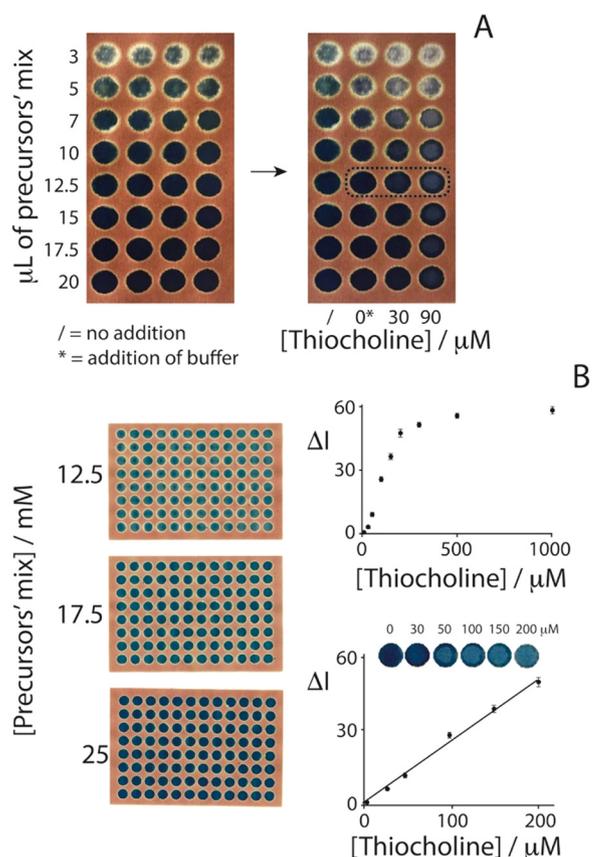


Fig. 3. A) Optimization of the volume of precursors' mix to be loaded onto each paper-based well. The study was performed by varying the volume in the 3–20  $\mu\text{L}$  range, in the absence and in the presence of thiocholine (30 and 90  $\mu\text{M}$ ); B) Left: optimization of the concentration of PB-precursors in the 12.5–25 mM range. Right: calibration curve in the presence of thiocholine (up to 1 mM) obtained by using 25-mM PB-precursors.

the coffee ring effect. Once the selection of the precursors and sample volumes, the concentration of precursors was successively investigated. It should be considered that a high concentration of PBNPs, as the involved reaction scheme with thiocholine, could provide huge amount of

reactive sites but low concentrations could produce unperceivable color changes. However, even if low concentration of PBNPs would better visualize low levels of thiocholine, a low intensity of the blue-colored well is characterized by a narrow linearity range and by a limited repeatability due to the scarce homogeneity. The best outcomes in terms of homogeneity, sensitivity, and linear range were observed by considering the 12.5–25 mM range of precursors to synthesize PBNPs within the paper (Fig. 3B). The 96-well platforms were tested in the presence of thiocholine ranging from 25 to 1000  $\mu\text{M}$ . Moreover, the intensity of color, and its related changes, was quantified by adopting four different light-mode: green, red, blue and grey. 25-mM precursors were selected for carrying out the development of the platform, because this generates a homogeneous PBNPs layer and a higher repeatability and sensitivity. In addition, the fading in the presence of buffer was not remarkable at this PBNPs level. The green mode, with respect to the others, was consistent with higher sensitivity:  $y = 0.27x - 1.63$  ( $R^2 = 0.991$ ),  $y = 0.22x - 5.72$  ( $R^2 = 0.986$ ),  $y = 0.20x - 3.47$  ( $R^2 = 0.993$ ),  $y = 0.23x - 4.31$  ( $R^2 = 0.995$ ) (where  $y$  indicates the difference of color intensity,  $x$  indicates the concentration of thiocholine as  $\mu\text{M}$ ), respectively for green, red, blue, and grey mode. The platform showed a linearity up to 200  $\mu\text{M}$  with a detection limit of 11  $\mu\text{M}$  (calculated as 3 times the standard deviation of the blank solution divided by the slope of the calibration curve).

### 3.1.1. Evaluation of the repeatability of platform

The analytical measurement of the 96-well paper-based platform should be characterized by a good repeatability that is dependent on the route of manufacture. Because of the paper acting as reactor for PBNPs synthesis, the substrate influences both the synthetic yield and the homogeneity of the final well. Being this method based on the change of color from PB to PW, the entire wells need to be characterized by small differences in terms of color intensities. The use of the chromatographic paper Whatman No.1, with respect a low-quality filter paper, i.e. Cordenons, was consistent in a very homogenous synthesis. The role of paper is to host all the precursors and activating them for PBNPs synthesis: Whatman No.1 is characterized by high-quality properties regarding the retention of fluids, thickness, ash content, etc. Both the intra- and inter-repeatability were evaluated. Regarding the intra-repeatability, it was calculated by taking three different 96-well platforms, and each multi-platform highlighted a relative standard deviation comprised between 1.1% and 4.0% (Supporting information, Fig. S1). Instead, the inter-repeatability calculated by considering all the three different platforms confirmed the high quality of PBNPs synthesis with low relative standard deviation values (ca. 3%, 288 wells).

### 3.2. Detection of BChE in standard solution

Being the physiological BChE activity comprised in the 5.9–13.2 U/mL range (Calderon-Margalit et al., 2006), the effectiveness of the multi-platform was preliminarily evaluated in this range. Before to detect BChE at various levels, it was evaluated how the presence of the enzyme, in the absence of its substrate (butyrylthiocholine), affected the fading of PBNPs compared with the only buffer solution (Supporting information, Fig. S2): results showed that the fading is ascribable only to the presence of the enzymatic by-product thiocholine, and not to the occurrence of just one between enzyme and substrate. However, in order to develop a reagent-free multi-platform, all the reagents are loaded (i.e. phosphate buffer, butyrylthiocholine) in each of the 96 wells. Consequently, the response was evaluated in function of butyrylthiocholine within the 10–500  $\mu\text{M}$  range, keeping the concentration of the enzyme equal to 10 U/mL as reported in Fig. 4A.

As observed in Fig. 4A, 200  $\mu\text{M}$  of butyrylthiocholine was consistent with the minimum amount (5  $\mu\text{L}$ ) of substrate to achieve high rate of enzymatic activity, thus this concentration was chosen for the monitoring of enzymatic activity. Therefore, each well of the paper-based

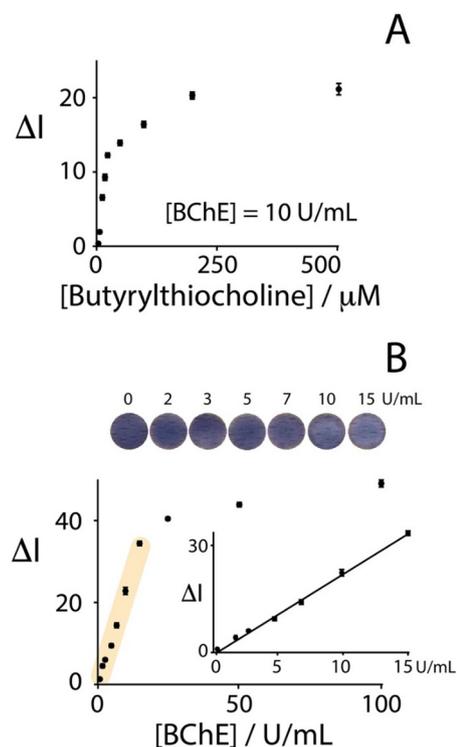
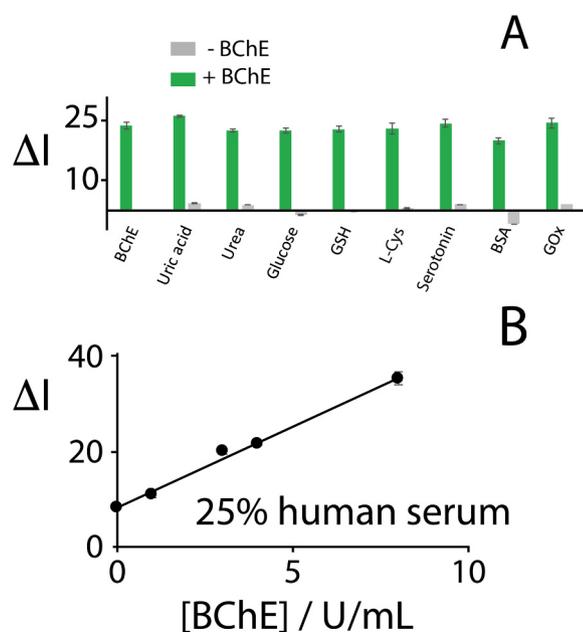


Fig. 4. A) Choice of the butyrylthiocholine concentration (10–500  $\mu\text{M}$ ) in the presence of 10 U/mL BChE; B) Calibration curve in the presence of 200  $\mu\text{M}$  butyrylthiocholine in the 0–100 U/mL range BChE.

platform was impregnated with 200  $\mu\text{M}$  of butyrylthiocholine previously dissolved in phosphate buffer (pH 7.4). The enzyme level was evaluated in the range comprised between 1 and 100 U/mL. As shown in Fig. 4B, a linear correlation was obtained up to 15 U/mL, and described by the following equation  $y = 2.37x - 1.40$  ( $R^2 = 0.995$ ), where  $y$  indicates the difference of color intensity and  $x$  indicates the U/mL of BChE. The limit of detection, calculated as 3 times the standard deviation of the blank solution divided by the slope of the calibration curve, was found equal to 0.8 U/mL. It should be noted that all the measurements were carried out simultaneously by scanning the 96-well paper-based platform after 25 min. The use of office scanner allows a better sensitivity in comparison with naked eye visualization: indeed, even if the discrimination in the 2–10 U/mL range might seem weak at the naked eye, the use of dark condition (as the office scanner provides) allows for a better discrimination between low concentrations of BChE, because of the absence of external light sources that can affect the visualization of fading. As reported in Supporting information (Fig. S3A), the fading due to the enzymatic reaction remained stable for 90 min after the first read (25 min are taken as time 0), and after 120 min just displayed a 7%-decrease. Moreover, the stability of the platform in the absence of enzyme was also evaluated: in the presence of loaded enzymatic substrate, the color of the wells remained constant up to 200 min (within the experimental errors), displaying a 15%-variation after 6 days' storage at room temperature, Fig. S3B (Supporting information). Some features of the herein reported method and other paper-based assays for BChE activity are listed in Table S1 (Supporting information).

### 3.3. Detection of BChE in human serum

After having highlighted the satisfactory analytical performances of the 96-well paper-based in standard solution, the activity of BChE was monitored in human serum samples. However, before to analyze human serum samples, the effect of some physiological interfering species was



**Fig. 5.** A) Interference study in the presence of ca. 0.6 mM uric acid, 17 mM urea, 28 mM glucose, 0.02 mM GSH, 0.2 mM L-Cys, 1  $\mu$ M serotonin, 0.7 mM BSA and 1 U/mL GOx. Each analysis was carried out in the absence (grey bars) and in the presence (green bars) of 10 U/mL BChE using 200  $\mu$ M butyrylthiocholine; B) Calibration curve obtained in 25%-human serum spiked with BChE at different levels.

investigated. The selectivity of the paper-based platform was evaluated measuring 10 U/mL BChE in the presence of uric acid, urea, glucose, glutathione (GSH), cysteine (L-Cys), serotonin, bovine serum albumin (BSA), and glucose oxidase (GOx). The interference concentrations were selected accordingly to the literature (Osawa et al., 2005; Cieplak et al., 2015; Liu et al., 2017; Godoy-Reyes et al., 2018; Huang et al., 2018). As shown in Fig. 5A, any relevantly affect for the visualization of BChE activity was observed in the presence of all interfering species tested, demonstrating the selectivity of the assay developed.

Taking into account the good selectivity of the multi-platform, the activity of BChE was monitored in a serum sample provided by a volunteer. As shown in Fig. 5B, a 25% dilution allowed to obtain a very good calibration curve described by the following equation  $y = 3.34x + 8.17$  with a  $R^2$  of 0.993. Moreover, with the standard addition method, the endogenous level of BChE was quantified to be ca. 10 U/mL that is in the physiological range (5.9–13.2 U/mL). Successively, one sample of human serum were used to evaluate the accuracy of the method by studying the recoveries. Serum was spiked, according with the linear range and detection limit of the method, in order to obtain concentration of BChE equal to 5 and 7 U/mL, and the recoveries were obtained, respectively, equal to  $113 \pm 3\%$  and  $102 \pm 4\%$  ( $n = 3$ ).

Besides these good results, the outcomes of the 96-well paper-based device in serum were also compared with those obtained using a standard spectrophotometric method in collaboration with the Department of Experimental Medicine, which provided four serum samples to be analyzed (Supporting information, Fig. S4).

The reference method is based on the spectrophotometric detection using butyrylthiocholine as substrate. In detail, in the presence of the enzyme the butyrylthiocholine is hydrolyzed to butyric acid and thiocholine, and the latter one is able to reduce ferricyanide to ferrocyanide, with a consequent decrease of the absorbance. This decrease is directly proportional to the enzyme activity in the serum sample, furnishing a quantitative and accurate measurement of BChE activity. This automated system (i.e. ARCHITECT System (Abbott)) allows for a linear range of 0.164–25 U/mL with a detection limit of 0.164 U/mL.

**Table 1**  
Comparison between 96-well visual platform and reference method for BChE activity measurement in serum.

Serum	Spectrophotometric method (U/mL)	96-well visualization (U/mL)	Accordance
#1	12.4	$10.5 \pm 0.6$	$85 \pm 5\%$
#2	6.3	$5.8 \pm 0.3$	$92 \pm 5\%$
#3	1.3	ND <sup>a</sup>	–
#4	1.4	ND <sup>a</sup>	–

<sup>a</sup> Not detected.

The results of the novel paper-based platform were compared with this reference method and are reported in Table 1.

The serum samples were detected by diluting the sample by using two different dilution factors 25% and 50%, exploiting the multiplex approach of this paper-based platform, to quantify the BChE over the linear range reported in Fig. 5B. For serum sample #1 and #2, a good accordance was observed. In the case of serum #3 and #4, BChE level were not detectable (ND) because the concentration is under the detection limit, considering the dilution factor (25%). The “ND” results have, however, a diagnostic screening value: the occurrence of BChE out of its physiological range, unequivocal indicates the need for more accurate clinical tests, thus reducing the analysis cost/time in case of healthy patients. Furthermore, to demonstrate the good precision of the sensor developed, the F-test was applied to sample #2, using the variance reported for the reference method and the variance obtained with the sensor (measurements carried out in triplicate). We found F value equal to 16.7, which is lower than the value reported in F-test table at 95% confidence level (i.e. 19), demonstrating the comparable precision among these two methods.

#### 4. Conclusions

For the first time, the effectiveness of paper-based fabrication, nanomaterials, and multi-visualization, has been satisfactorily combined for butyrylcholinesterase activity detection in human serum samples. The chromatographic paper employed for the realization of the platform demonstrated its suitability: i) in combination with wax printing, to fabricate 96 wells multi-platform, ii) to be actively used as reactor for Prussian Blue nanoparticle synthesis, iii) as the detector for BChE visualization, and iv) to load all the reagents for a zero-task enzyme visualization at the end-user stage. This novel approach takes advantage of the fading of Prussian Blue, which turns colorless in the presence of the enzymatic by-product thiocholine as a consequence of a redox reaction. The great advantage of this paper-based platform is related to the possibility to carry out, simultaneously, 96 experiments together within the same cost-effective device. Moreover, being the detection based on a clear color fading (blue to colorless), the visualization might be easily performed with a common office scanner, or with a smartphone setting (coupled with a 3D printed-cartridge for light insulation). A facile fabrication and equipment allows for very repeatable device (relative standard deviation of ca. 3%,  $n = 288$ ), successfully applied for monitoring BChE activity within physiological range in human serum samples. Furthermore, the entire paper-based nature of this multi-platform, which does not include any metal-based inks, allows for reducing the issue of waste accumulation especially in limited-settings zones: the sustainability of paper is also related to the possibility of being easily burned after use. This approach might represent the starting point towards the realization of multi-analysis tools, allowing for accurate diagnoses and therapeutic interventions tailored to the individual patient in the field of precision medicine, as in the case of administering the assumption of cholinesterase inhibitors for treating Alzheimer's disease.

### CRedit authorship contribution statement

**Neda Bagheri:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Stefano Cinti:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Veronica Caratelli:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Renato Massoud:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Mohammad Saraji:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Danila Moscone:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing. **Fabiana Arduini:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - review & editing.

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### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bios.2019.03.037](https://doi.org/10.1016/j.bios.2019.03.037).

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