Modern creatinine (Bio)sensing: Challenges of point-of-care platforms

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ABSTRACT

The importance of knowing creatinine levels in the human body is related to the possible association with renal, muscular and thyroid dysfunction. Thus, the accurate detection of creatinine may indirectly provide information surrounding those functional processes, therefore contributing to the management of the health status of the individual and early diagnosis of acute diseases. The questions at this point are: to what extent is creatinine information clinically relevant?; and do modern creatinine (bio)sensing strategies fulfil the real needs of healthcare applications? The present review addresses these questions by means of a deep analysis of the creatinine sensors reported in the literature over the last five years. There is a wide range of techniques for detecting creatinine, most of them based on optical readouts (20 of the 33 papers collected in this review). However, the use of electrochemical techniques (13 of the 33 papers) is recently emerging in alignment with the search for a definitive and trustworthy creatinine detection at the point-of-care level. In this sense, biosensors (7 of the 33 papers) are being established as the most promising alternative over the years. While creatinine levels in the blood seem to provide better information about patient status, none of the reported sensors display adequate selectivity in such a complex matrix. In contrast, the analysis of other types of biological samples (e.g., saliva and urine) seems to be more viable in terms of simplicity, cross-selectivity and (bio)fouling, besides the fact that its extraction does not disturb individual's well-being. Consequently, simple tests may likely be used for the initial check of the individual in routine analysis, and then, more accurate blood detection of creatinine could be necessary to provide a more genuine diagnosis and/or support the corresponding decision-making by the physician. Herein, we provide a critical discussion of the advantages of current methods of (bio)sensing of creatinine, as well as an overview of the drawbacks that impede their definitive point-of-care establishment.

1. Introduction

Creatine is mainly synthesized in the kidneys, liver and pancreas (Fig. 1a) before being transported to the tissues and organs, where it is metabolized to creatinine (Fig. 1b), termed CRE. When adenosine triphosphate is involved in this conversion, creatine per se is a source of energy for many biological processes, such as muscle activity (Narayanan and Appleton, 1980). In this context, the creatine to CRE cyclization rate is not fully understood despite this knowledge potentially assisting in the control of degenerative diseases of the muscles as well as improvement of sport performance (Diamond, 2005). In contrast, it is well-known that CRE levels are fairly constant in the human body, mainly depending on the muscle mass of the individual (Narayanan and Appleton, 1980; Pundir et al., 2013). For example, the typical reference ranges for serum levels of CRE in healthy patients are on the order of 45–90 μM for women and 60–110 μM for men (see Table 1; Randviir and Banks, 2013). After subsequent CRE generation in

the muscles and other parts of the body, CRE is transported through the bloodstream by the kidneys and excreted in the urine. Accordingly, both fluids (blood and urine) deserve clinical attention with respect to CRE levels (Killard and Smyth, 2000).

CRE is the second most analysed biomolecule for clinical purposes after glucose (Joffe et al., 2010). Thus, CRE values outside of typical ranges is evidence of any health issue associated with renal, muscular and thyroidal functioning; and levels fairly beyond healthy ones encompass very serious diseases, such as chronic kidney disease (CKD), different types of muscular disorders, cardiovascular problems or even Parkinson's disease, among others. As an example, when CRE levels in the serum (i.e., blood) are below 40 μM (Table 1), this indicates an abnormal reduction in muscle mass (Killard and Smyth, 2000). However, for concentrations greater than 150 μM (Table 1), additional analytical tests are required to exclude any risk of CKD. In extreme cases, values above 500 μM inform of a clear renal impairment that will likely involve dialysis treatment or kidney transplantation (Killard and
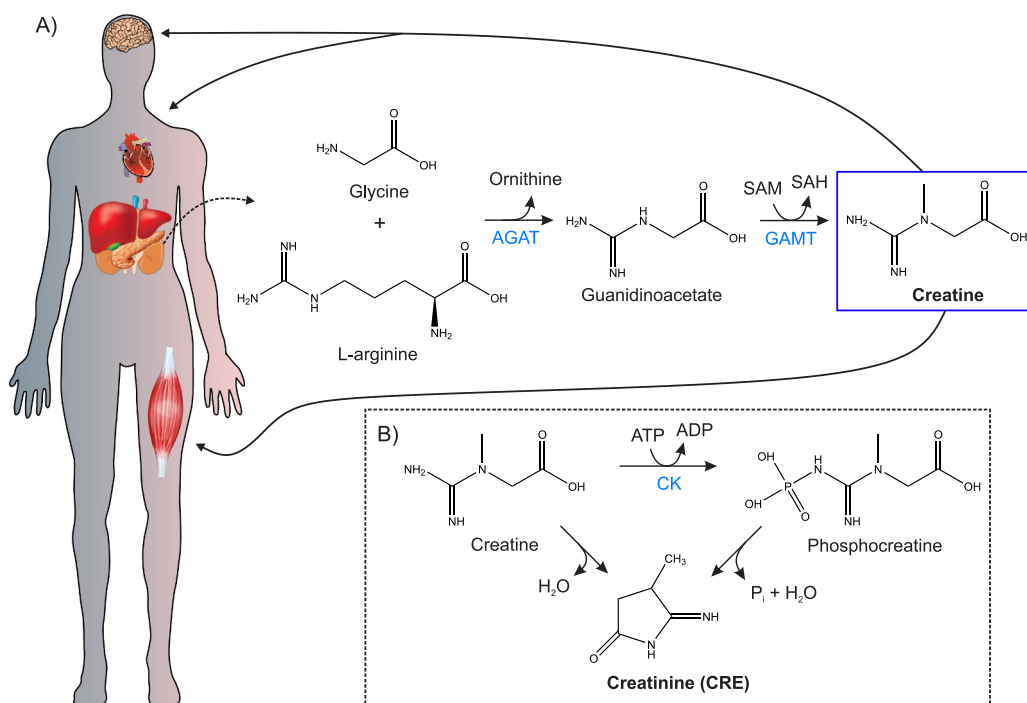
Fig. 1. General scheme of the metabolism of Creatine to CRE. Creatine originated in different organs is metabolized into CRE in muscles and brain. a) Illustration of the synthetic route of Creatine by the organism together with the main organs in which these processes take place (kidneys, liver and pancreas). AGAT: L-arginine: glycine amidinotransferase and GAMT: S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase. Black arrows show where Creatine is required as a source of energy (producing CRE), mainly brain and muscles. b) Creatine metabolization to CRE through hydrolysis reactions or involving phosphocreatine as an intermediate. CK: Creatine kinase; biosynthesis of creatinine occurs during the reaction.

Table 1
Ranges of CRE levels found in different matrices from healthy and unhealthy individuals.

Sample	pH range[a]	Healthy levels [CRE]	Harmful levels [CRE][a]
Urine	4.5 – 8[b]	4.4 – 18 mM[c]	< 3 and > 20 mM[c]
Serum / Blood	7.35–7.45	45 – 90 μM (♂) 60 – 110 μM (♀)[d]	< 40 μM and > 150 μM (up to 1 mM)[d]
Saliva	6.2–7.4	4.4–17.7 μM[e]	17.7 up to 591 μM[e]
Sweat	4–6.8	9.4–18 μM[f]	60 – 200 μM[f]
Tears	6.5–7.5	–	–
ISF	7.2–7.4	[g]	[g]

[a] (Corrie et al., 2015).
[b] The range of pH can vary also depending on the different laboratories and, in the case of urine, it can be even wider in case of infection.
[c] (Li et al., 2015; Ruedas-Rama and Hall, 2010).
[d] (Killard and Smyth, 2000; Tseng et al., 2018).
[e] (Lloyd et al., 1996; Venkatapathy et al., 2014).
[f] (Al-Tamer et al., 1997; Bass and Dobalian, 1953). The symbol ♂ indicates males and ♀ in females.
[g] Similar levels of CRE can be found in ISF and serum (Ebah, 2012; Kayashima et al., 1992).

Smyth, 2000). Furthermore, persistently elevated levels of CRE may reveal a high risk of mortality (Levey et al., 2015). Notably, the interpretation of CRE observations must be always carried out considering the muscle mass of the patient as the same value may be considered normal in a young male with a relatively high muscle mass or may indicate CKD in elderly females (Tseng et al., 2018).

There are a number of organizations, such as the American Association of Kidney Patients, American Kidney Foundation, National Kidney Foundation in New York and the Nephron Information Center, among others[1] that define five different stages for kidney function based on the estimated glomerular filtration rate (eGFR), which in turn is calculated from CRE levels in serum together with other factors, such as gender and age. In addition, CRE clearance is computed from CRE

serum measurements, as well as the albumin-to-CRE ratio in urine to evaluate the state of kidney activity (Cockcroft and Gault, 1976; Junge et al., 2004; Levey et al., 2015; Omoruyi et al., 2012; Tziakas et al., 2015). The early diagnosis of any sort of dysfunction is extremely important in the prevention and/or control of CKD because this illness is asymptomatic during the first stages (mild and moderate CKD). Hence, when the individual notices any symptom, the disease is likely very advanced, and the kidneys are commonly quite damaged. Altogether, the patient experiences a radical change in their life because CKD treatment involves visiting the hospital every two days for dialysis or, if the damage is too severe, that person would need a kidney transplant. Importantly, over one million people worldwide are undergoing dialysis treatment. Moreover, the incidence of renal failure has doubled over the last 15 years (Bagalad et al., 2017). Therefore, there is a societal need for trustable detection of CRE at the point-of-care (POC) level not only to allow one to preserve individual well-being and contribute to a more affordable healthcare system, but also to monitor patients at advanced stages of CKD, which necessitates more than one analysis per day during dialysis treatment (Hannan et al., 2014; Michalec et al., 2016).

CRE detection is also crucial in the premature diagnosis of various muscular diseases, such as Duchenne muscular dystrophy (Fitch and Sinton, 1964), myasthenia gravis, acute myocardial infarction (Radomska et al., 2004b) or guanidinoacetate methyltransferase (GAMT) deficiency (Diamond, 2005), as well as verifying patient status before and after surgical interventions (Ho et al., 2012; Prowle et al., 2014; Spahillari et al., 2012; Vart, 2015) or when suffering an accident, as muscular lesions are somewhat related to higher concentrations of CRE in the bloodstream. Besides this, CRE detection is valuable for verifying dehydration status in individuals, e.g., as a consequence of a decrease of renal blood flow during or after engaging in strong physical activity (Baxmann et al., 2008; Mohamadzadeh et al., 2016). In addition, the administration of certain drugs and/or treatments, such as acetylcholine inhibitors, cyclosporin or chemotherapy, may present side-effect damage in the kidneys, and therefore, high levels of CRE are probably observed owing to elevated renal impairment (Cherney et al., 2017; Kulling et al., 1995; Wiebe et al., 2017). In all these cases, POC detection of CRE should supply a real-time analysis that yields clinical information regarding renal functionality before, during and after

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[1] www.aakp.org; www.kidneyfund.org; www.kidney.org; www.nephron.com.

therapy or physical activity.

Despite the proven necessity of CRE detection at the POC level as mentioned before, currently, analysis is always carried out in centralized laboratories after extracting the sample. In contrast, the definitive clinical analysis of CRE (at the POC level) must provide the features proposed in the ASSURED guidelines (St John and Price, 2014): the detection has to be Affordable, Sensitive (minimal false negatives), Specific (minimal false positives), User-friendly (simple enough to be conducted by the patient in remote settings), Rapid and Robust (avoiding hospital wait times and providing reliability), Equipment-free (or minimal equipment needed) and easily Delivered (to the end user) (Wang et al., 2016; Zarei, 2017). The present review critically analyses the reported (bio)sensors from 2014 as alternative approaches for the current analysis of CRE carried out in hospitals and clinical laboratories. For this purpose, it is important to establish in which biological samples CRE is present and what kind of clinical information is to denote its detection in each particular fluid.

Previous reports on CRE detection have collected papers published up to 2013 mainly describing CRE sensing concepts that were essentially classified into optical methods and biosensors based on enzymatic reactions (Lad et al., 2008; Killard and Smith, 2000; Shephard, 2011; Mohabbati-Kalejahi et al., 2012; Randviir and Banks, 2013; Pundir et al., 2013). Nevertheless, while CRE biosensing was claimed as the most promising alternative to substitute the well-established Jaffé method, other techniques also showed analytical features suitable for the clinical analysis of CRE. In this sense, Lad et al. (2008) reported on analytical characteristics and several designs of electrochemical biosensors based on different recognition elements such as enzymes, antibodies and molecular imprinted polymers (MIPs). Killard and Smith discussed on the advantages and limitations of potentiometric and amperometric biosensors based on enzymatic CRE recognition published up to 2000. Shephard (2011) reviewed all the POC devices available for measuring CRE in whole blood, serum and plasma up to 2011. The review by Mohabbati-Kalejahi et al. (2012) highlighted that chromatographic CRE analysis provided improved limit of detection (in the order of 0.28 nM) while potentiometric electrodes presented fast response time and those based on MIPs displayed the best selectivity. Randviir and Banks (2013) summarized the analytical methodologies (enzymatic and non-enzymatic) utilized to quantify CRE up to 2013. Pundir et al. (2013) published a work focused on the status of enzymatic and non-enzymatic electrochemical CRE detection as well as certain immunosensors and the introduction of nanomaterials towards the development of smart sensing devices. Beyond providing an updated collection of published papers on CRE (bio)detection from 2014 up to the present, this review 'puts on the table' for the first time the advantages of modern CRE (bio)sensing together with the analytical drawbacks that impede definitive POC establishment.

2. In which human fluids is it plausible to determine creatinine to obtain clinically relevant information?

CRE is present in a variety of biological fluids (Table 1) because of its participation in diverse metabolic routes, as already described in the introduction (Narayanan and Appleton, 1980). As a result, the analysis of CRE in distinct samples provides different types of clinically relevant information. For example, CRE detection is already included in routine blood analysis as a preliminary check for the malfunction of kidneys. Typically, CRE detection is accomplished in clinical laboratories after blood extraction. Note that centralized measurements are the opposite to the ASSURED requirements to establish a POC diagnosis platform (St John and Price, 2014). On the other hand, CRE analysis in urine is also very common, which is performed during a 24 h sample collection to provide more accurate results (see more details subsequently). Both blood and urine analysis are based on the Jaffé method (Greenberg et al., 2012).

The Jaffé method involves a colorimetric readout of the sample

when picric acid interacts with CRE to form a chromogen that absorbs within the wavelength range of 470–550 nm (red colour) with a normal maximum at ca. 520 nm (Mohabbati-Kalejahi et al., 2012). Despite this reaction being nonspecific for CRE, in fact, the method's sensitivity is affected by temperature, pH and others variables (Walsh and Dempsey, 2002); the Jaffé method is still widely used owing to the rapid analysis (~15 min per sample), its cost-effectiveness and the fact that it is adaptable for automatization (Narayanan and Appleton, 1980; Spierto et al., 1979). Importantly, CRE concentrations determined by this method must be carefully interpreted because of a number of reasons:

(i) The reaction is extremely sensitive to temperature, e.g., a variation of 1 °C produces an error of ± 0.7 μM in the estimated CRE concentration, which can lead to erroneous decision-making in cases of a potential decrease in muscle mass (Narayanan and Appleton, 1980; Spierto et al., 1979).

(ii) The risk of sample contamination is relatively high in the case of blood analysis as colorimetric detection cannot be performed directly in blood because of two main reasons. First, the red colour of the blood impedes the detection of the chromogen adduct in the red band. Second, proteins and other compounds strongly interfere in the derivation reaction. Consequently, the technique is applied to serum and not whole blood (Doolan et al., 1962; Owen et al., 1954).

(iii) Spectrophotometric analysis generally requires a sample volume from 50 μL to 2 mL depending on the instrumentation available in the laboratory (Walsh and Dempsey, 2002). Indeed, certain spectrophotometers operate with volumes of just a few microliters (0.5 – 5 μL).[2] There are also commercially available kits with the required amount of picric acid for specific sample volume[3]. In other cases, a sample dilution is normally needed to provide a CRE concentration within the linear range of response of the calibration. For instance, in the case of urine, a 5 – 20 dilution factor is traditionally demanded while even larger dilutions (400–800 fold) could be needed.[3] During serum analysis, this is less common. However, the total volume of blood or urine collected from the individual depends on the final aim and type of whole analysis, i.e., the nature and range of parameters to be analysed in the same sample. In the case of urine, as the CRE levels can fluctuate depending on diet, hydration and other factors (Boeniger et al., 1993), a single spot check is insufficient for decision-making and therefore, the average content in urine collected over 24 h is preferable in terms of an accurate clinical indication. Overall, the required blood and urine collection as well as manipulation is considered tedious and risky, and the dilution requirement will inherently involve an error in CRE concentration estimation (Narayanan and Appleton, 1980).

(iv) Regarding CRE levels in urine, this is normally expressed as CRE clearance by kidneys, which can be estimated following two approaches (Doolan et al., 1962). One is based on blood measurements ($[CRE]_{serum}$ = CRE mg in 100 mL of blood) and employs the Cockcroft-Gault formula that considers a fixed value for patient's gender (72 for males and 85 for females; in the latter, the number 72 in Eq. (1) has to be replaced), weight and age (Eq. (1)) (Cockcroft and Gault, 1976).

$$CRE_{clearance} = \frac{(140 - age) \times weight}{72[CRE]_{serum}} \tag{1}$$

The second approach consists of a comparison between the average CRE levels excreted in 24-h urine by the individual and the blood content after this collection time (Mohabbati-Kalejahi et al., 2012;

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[2] www.metrixlab.mx

[3] https://www.alpco.com,   www.mybiosource.com,   www.abcam.com, www.sigmaaldrich.com

Narayanan and Appleton, 1980; Schwartz et al., 1987). In principle, the latter should provide more accurate results because it considers the CRE content in two different biological fluids rather than an empirical formula (Cockcroft and Gault, 1976).

(v) While the Jaffé method is considered the gold standard for CRE detection, it normally provides CRE levels slightly higher than other analytical techniques (ca. 3–5%). Indeed, extreme differences (even greater than 10%) were reported for some isolated cases involving HPLC and amperometric techniques (Walsh and Dempsey, 2002). This fact has to be considered as another warning for the careful clinical interpretation of analytical observations. Besides, it is important to consider the toxicity and explosive issues of picric acid, which additionally require expertise by the end user (He et al., 2015).

(vi) Biological samples have different pH values (Table 1) and this has to be considered when applying the Jaffé method for comparative purposes as the ratio of alkaline picrate and alkaline CRE picrate varies with pH, therefore influencing the final colour of the sample (Narayanan and Appleton, 1980; Owen et al., 1954).

It is worth mentioning that there is additional clinical information that can be drawn from blood and urine analysis, such as the urea nitrogen-to-creatinine ratio in blood as well as the potassium-to-creatinine and albumin-to-creatinine ratios in urine (BUN-CRE, K-CRE and A-CRE ratios, respectively) (Bartz et al., 2015; Lin et al., 2009). Once the BUN-CRE ratio is higher than normal (from 10:1–20:1), this may indicate the malfunction of the kidneys or presence of stones or tumours (Winarta et al., 2015). Moreover, once the BUN-CRE ratio is too high, severe conditions consisting of kidney failure, shock or hydration can arise (Lin et al., 2017). Conversely, when the ratio is too low, this can be caused by malnutrition, poor diet in terms of protein or severe liver damage (Winarta et al., 2015).

The simultaneous analysis of CRE together with potassium or albumin in urine collected for 24 h or as one-spot may also have clinical usefulness. For example, if the K-CRE ratio is greater than 1.5, this is indicative of renal potassium waste because of any organism dysfunction (Jędrusik et al., 2017). In this context, Assadi et al. reported a valuable study based on specific clinical cases, showing what type of diseases can be caused by various metabolic conditions together with the K-CRE ratio (Assadi, 2008). In addition, different organizations (such as the American Diabetes Association, International Diabetes Federation, National Kidney Foundation, Caring for Australians with Renal Impairment, UK Renal Association Clinical Practice Guidelines for the Care of Patients with CKD, among others) recommend the utility of the A-CRE measurement when a 24-h urine collection period is not available. The concentration considered the 'decision limit' reported by laboratories varies from 15 to 30 mg/L for urine albumin and 1.0–3.6 mg/mmol (9–32 mg/g) for A-CRE (Miller et al., 2009). Risk for both cardiovascular and kidney disease rises significantly with urine ACR above 10 mg/g (Kramer et al., 2017). Moreover, A-CRE can be valuable for predicting acute kidney injury during hospitalization for acute myocardial infarction (Tziakas et al., 2015), cardiovascular events and diseases in adults (Bartz et al., 2015; Cho et al., 2015), as well as early detection of CKD (Omoruyi et al., 2012). Nonetheless, A-CRE varies depending on the age, gender and ethnicity, and therefore, medical decision making should be carefully undertaken (Marcovecchio et al., 2018).

One advantage of using urine versus blood is the easy sample collection that generally confers no disturbance to patient integrity, unlike with needle-based extractions. However, overall and in spite its complexity, blood offers the most rapid and clinically relevant detection of CRE (Peake and Whiting, 2006). Nevertheless, the use of other biomatrices is gaining more and more interest with respect to non-invasive analysis beyond urine. However, this trend has not yet been outlined in (bio)sensors reported over the last five years for CRE detection (see Table 2 and the next section).

Saliva is generally a robust estimator of what takes place in the blood. There are a variety of well-known correlations in terms of relevant parameters extracted from saliva that may take the place of analysing them directly in blood, e.g., proteins, biomarkers and ingested drugs, among others (de Almeida et al., 2008; Corrie et al., 2015; Lee and Wong, 2009; Maccallum and Austin, 2000; Pfaffe et al., 2011). Indeed, saliva has been demonstrated as an alternative to monitor metabolic by-products of kidney failure (Lloyd et al., 1996; Nagler, 2008). For example, CKD is one of the systemic diseases that can affect the contents of salivary secretions (Lasisi et al., 2016). It has been reported that CRE enters saliva via ultrafiltration, and its salivary concentration seems to range from 10% to 15% of that found in blood (Lloyd et al., 1996). Whereas studies have shown promising results based on the comparison of CRE levels detected in saliva and serum samples, for instance, accuracy in the range of 90–95% (Bagalad et al., 2017; Venkatapathy et al., 2014), saliva analysis has less diagnostic accuracy and specificity versus traditional colorimetric detection of CRE in serum (Xia et al., 2012; Lasisi et al., 2016). However, these results could be influenced by the inherent error associated with the employment of the Jaffé method as well as all the steps involved in sample collection and storage (Bagalad et al., 2017).

Even though the use of saliva in diagnostic tests has many advantages (i.e., non-invasive and affordable collection in terms of complexity and costs, appropriateness for all age groups, convenience for use in non-laboratory areas and the screening of large population (Pfaffe et al., 2011), suitability for patients suffering from clotting disorders and with compromised venous access (Gröschl, 2008; Lee and Wong, 2009; Nagler, 2008; Venkatapathy et al., 2014)), it is important to remark that salivary concentrations of certain compounds can be affected by many factors, not only including gender, but also diet and genetics (Fahed et al., 2012; Liu et al., 2013). As a result, the utility of saliva as a general diagnostic fluid is still subject to continuous empirical evaluation (Lasisi et al., 2016). While serving as a promising candidate for POC detection of CRE, there is plenty of room for investigation in this direction, especially considering the accuracy of the results, noting that only 18 of the 33 papers presented in Table 2 have been validated with a standard method, and their translation into trustworthy clinical decision-making.

Another alternative is the use of sweat, which is being accounted for in the development of many wearable sensors at present (Bandodkar et al., 2016; Florea and Diamond, 2015; Parrilla et al., 2018a). Collection of sweat is also non-invasive, but typically provides considerably less sample volume than saliva. Moreover, there is one inconvenience associated with the procedure of collecting samples based on exposing the patient to high temperatures to facilitate an enhanced sweating rate. For example, when patients are elderly, they may experience side effects related to exposure to high temperatures, such as low blood pressure or dizziness. In addition, an inevitable evaporation during the collection is generally translated into overestimation of CRE levels (Al-Tamer et al., 1997).

Eccrine sweat fluid has been shown to contain several biochemical markers of clinical interest (Sato et al., 1989), and comprises the natural route for the excretion of biological waste products, as is the case when kidneys are damaged and their function compromised (Al-Tamer et al., 1997). Nonetheless, early evidence has suggested that CRE content in sweat is closely linked to the rate of sweating rather than with the level of CRE in blood (Ladell, 1947). Hence, the slower the rate of sweating, the higher the CRE content. Additional studies have revealed how the rise in CRE content in the blood is correlated with an increase of CRE in saliva, but varies slightly with respect to sweat content. This fact may likely impede the selection of sweat as a fluid for CRE detection, otherwise at least a constant sweating rate is achieved by all individuals, and therefore, all results may be standardized.

Another biological fluid considered for CRE detection is tears. Despite the high variety of compounds present in this fluid (electrolytes, organic salts, proteins, glucose, urea and other biopolymers)

Table 2
Summary of the CRE (bio)sensors reported in the literature from 2014 up to now.

Readout	Description	Sensing principle	Working range	LOD	Analysis Time	Validation	Sample	POC	Ref.
Colorimetry	Paper-based chip with CMOS camera and USB connection	Reaction with picric acid / NaOH (Jaffé)	17.7 – 707 µM	–	5 min	Correlation R^2 = 0.9994	Serum	No[a]	Fu et al. (2018)
Colorimetry	µPAD with CMOS camera and Wifi chip	Reaction with picric acid / NaOH (Jaffé)	16.8 – 675 µM	16.8 µM	8 min	Correlation R^2 = 0.9920	Whole blood	Yes	Tseng et al. (2018)
Colorimetry	AgNPs-based sensor	Alkaline CRE aggregation with citrate-capped AgNPs	0 – 4.2 µM	53.4 nM	1 up to 10 min	–	Diluted urine	No	Alula et al. (2018)
Colorimetry	Photosensor based on LEDs and LCD screen	Enzyme cascade (BIOSENSOR)	67.2 – 1768 µM	60 µM	5 min	–	Plasma	Yes	Dal Dosso et al. (2017)
Colorimetry	AuNPs-based sensor	Synergistic adenosine/CRE coordination	–	12.7 nM	< 5 min	–	Artificial urine Bovine serum	No	Du et al. (2016)
Colorimetry	AgNPs-based sensor	AgNPs coated with picric acid (Jaffé)	0.01 – 1 µM	8.4 nM	4 min	–	Serum CSF	No	Parmar et al. (2016)
Colorimetry	Paper-based strips	Enzyme cascade (BIOSENSOR)	0.22 – 2.21 mM	0.17 mM	11 min	Correlation R^2 = 0.977	Urine	No	Talalak et al. (2015)
Colorimetry	CDS with camera and CSPT array	Jaffé	160 µM – 1.6 mM	89 µM	5 – 15 min	Correlation R^2 = 0.930 – 0.977	Diluted urine	No	Debus et al. (2015)
Colorimetry	AuNPs in solution	Cross-linking reaction	0.1 – 20 mM	80 µM	24 min	–	Diluted urine	No	He et al. (2015)
Colorimetry	plasmonic nanoparticles	CRE with uric acid and AuNPs modified with Hg^{2+}	0.1 – 0.4 mM	19.8 nM	> 5 min	Recovery 94 – 103%	Diluted urine	No	Du et al. (2015)
Colorimetry	Label-free AuNPs system	SPE of CRE with AuNPs	0.13 – 0.35 mM	0.12 mM	10 – 30 min	–	Diluted urine	No	Sittiwong and Unob (2015)
Absorbance / Fluorescence	Chemodosimeter	PTP chalcone	0.1 nM – 1.4 mM	0.058 nM	10 min	Recovery 88 – 105%	Serum	No	Ellairaja et al. (2018)
Optical (Liquid crystals)	DMOAP-coated glass slides	Hydrolysis of CRE with deprotonation of HBA (change in orientation of LCs)	–	as low as 50 µM	3 – 30 min[b]	–	Artificial samples	No	Verma et al. (2016)
Fluorescence (light-up)	Pd^{2+} naphthalimide-based fluorescence probe	Synthetic route leading to a degradation complex	–	as low as 0.30 nM	30 min	Recovery 5 – 99%	Serum	No	Pal et al. (2016)
ECL	ITO electrode with NiNCs	Magnetic graphene oxide (GO-Fe_3O_4) in MIP	5 nM – 1 mM	5 nM	15 – 30 min	Recovery 93 – 100%	Diluted serum Diluted urine	No	Babamiri et al. (2018)
PL	carbon nanodots	BSA + CRE	17 µM – 1.7 mM	6.2 µM	5 min	–	Artificial samples	No	Babu et al. (2018)
CL	Co^{2+}-based system	CRE + H_2O_2	0.1 – 30 µM	72 nM	1 min	Recovery 100 – 103%	Diluted urine	No	Hanif et al. (2016)
SPR	gold grating substrate	AgNPs deposited with PDADMAC and PSS	0.1 – 20 mM	–	> 10 min	–	Artificial samples	No	Pothipor et al. (2017)
S-SERS	NPGD plasmonic substrates	CRE interaction with NPGD	100 nM – 100 µM	13.2 nM (in water)	Ca. 1 h	–	Artificial samples	No	Li et al. (2015)
IMS	PPy/GO fibres	SPME with fast evaporation	0.01 – 4.4 mM 0.02 – 2.2 mM	5.3 µM 22.9 µM	7 – 10 min	Recovery 92 – 104% and 101 – 110%	Spiked diluted urine and plasma	No	Jafari et al. (2015)
CV	Carbon ink screen printed electrodes	Complex formation with cooper	6 – 378 µM	0.07 µM	> 30 min	Recovery 95 – 98%	Serum	No	Raveendran et al. (2017)
CV	PINE electrodes coated with $FeCl_3$ cotton membranes	FeIII binding	0.88 – 22 mM	–	< 1 min	Correlation R^2 = 0.91	Urine	Yes	Kumar et al. (2017b)
CV and DPV	Graphite electrode	CdSe semiconductor QDs	0.44 µM – 8.84 mM	0.23 µM	Ca. 2 h	–	Urine Serum	No	Hooshmand and Es'haghi (2017)
CV and DPV	MWCNT screen printed electrode	Enzymatic recognition by papain (cysteine protease) (BIOSENSOR)	–	0.006 nM	10 min	–	Spiked urine	No	Desai et al. (2018)
DPV	GC electrode with Ni-PANI NPs	Magnetic molecularly imprinted polymer	0.004 – 0.8 µM	0.2 nM	Ca. 3 h	Recovery 95 – 102%	Artificial sample	No	Rao et al. (2017)
DPV	Screen printed gold electrode	Molecularly imprinted polymers (MIPs)	0.88 – 8.84 nM	0.14 nM	2 – 5 h	Correlation R^2 = 0.99	Diluted urine	No	Diouf et al. (2017)
DPV	Magnetic GC electrode with Fe_3O_4-PANI NPs	Magnetic molecularly imprinted polymer	0.02 – 1 µM	0.35 nM	Ca. 55 min	Recovery 90 – 105%	Artificial samples	No	Wen et al. (2014)
Amperometry	PINE electrode	$FeCl_3$ electroactivity	17.6 – 512.7 µM	–	> 10 min	–	Saline media	No	Dasgupta et al. (2018)

Table 2 (continued)

Readout	Description	Sensing principle	Analysis Time	LOD	Working range	Validation	Sample	POC	Ref.
Amperometry	Enzyme NPs immobilized in GC electrode	Enzyme cascade (BIOSENSOR)	2 s	0.01 μM	0.01 – 12 μM	Correlation R² = 0.99	Diluted serum	No	Kumar et al. (2017a)
Amperometry	SPE with Cu, Nafion, PANI and the enzyme	Enzymatic reaction (BIOSENSOR)	15 s	0.5 μM	1 – 125 μM	Correlation R² = 0.99	Diluted serum	No	Zhybak et al. (2016)
ISFET	Zeolites based electrodes and AuNPs	Enzymatic reaction (BIOSENSOR)	1 – 4 min	5–10 μM [e]	Up to 2 mM	–	Artificial sample	No	Ozansoy et al. (2017)
Potentiometry	GC electrode with selective membrane	Selective recognition of creatininium cations with ionophore	1 min	0.63 μM (urine)	1 μM – 10 mM; 0.3 – 0.6 mM	Correlation R² = 0.966	Diluted urine; Diluted plasma	No	Guinovart et al. (2016, 2017)
Conductometry	Interdigitated electrode with contact printing based enzymatic membrane	Enzymatic recognition (BIOSENSOR)	> 3 min	2 μM	10 – 600 μM	–	Artificial serum	No	Braiek et al. (2018)

CMOS = complementary metal oxide semiconductor; USB = universal serial bus; μPAD = microfluidic paper-based analytical device; AgNPs = silver nanoparticles; CRE = creatinine; LEDs = light-emitting diodes; LCD = liquid crystal display; AuNPs = gold nanoparticles; CSF = cerebrospinal fluid; CDS = circular dichroism spectroscope; CSPT = computer screen photo-assisted technique; SPE = solid phase extraction; PTP = (E) – 3-(pyren-2-yl) – 1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one; DMOAC = N,N-dimethyl-N-octade-cyl-3-aminopropyltrimethoxysilyl chloride; HBA = Hexylbiphenyl-4-carboxylic acid; LCs = liquid crystals; ECL = electrochemiluminescence; ITO = Indium-tin oxide; GO-Fe₃O₄ = graphene oxide – iron oxide nanoparticles; NiNCs = nickel nanoclusters; MIP = molecularly imprinted polymer; PL = photoluminescence; BSA = bovine serum albumin; CL = chemiluminescence; SPR = surface plasmon resonance; PDADMAC = poly (diallydimethylammonium chloride); PSS = poly (sodium 4-styrenesulfonate); S-SERS = stamping surface enhanced Raman scattering; NPGD = nanoporous gold disk; IMS = ion mobility spectrometry; PPy/GO = Polypyrrole/graphene oxide; SPME = Solid phase microextraction; CV = cyclic voltammetry; DPV = differential pulse voltammetry; QDs = quantum dots; MWCNT = multiwalled carbon nanotube; GC = glassy carbon; PANI = polyaniline; ISFET = Ion Sensitive Field Effect Transistor.

[a] "These human serum creatinine samples are obtained from whole blood samples via a separation and purification process at the hospital".
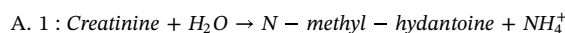
[b] This time can increase up to 3 h depending on the enzyme concentration as well as the CRE concentration.

(Zapata et al., 2005), large-sized biomolecules, like CRE, are found in very low concentrations likely because of solubility issues (Gaebler and Keltch, 1927; Sivapragasam et al., 2016). Some decades ago, Kang et al. (1988) presented the different contents of CRE found in blood and tears from 30 healthy control patients (mean values of 83 versus 80 μM, respectively) and 10 end-stage CKD patients, the latter in which CRE amounts in tears from pre- and post-haemodialysis treatment were compared. Interestingly, following haemodialysis treatment, CRE content decreased 36% in the case of tears and 48% in blood. Later, Zapata et al. (2005) confirmed the same in horses, where the mean concentration of CRE in tears was 9.6% lower than in serum. Overall, there is lack of information regarding CRE in tears, both healthy and harmful levels, and the correlation with blood content.

Cerebrospinal fluid (CSF) is another fluid that could be potentially considered for CRE detection. However, sample extraction involves a high level of intrusion in patients. Consequently, the use of CSF is not discussed in this section as it is not compatible with the POC concept. In contrast, CRE is also found in interstitial fluid (ISF) (Kost et al., 2000; Paliwal et al., 2013), which has recently been the target of certain wearable sensors that were created with the goal of composition characterization (Parrilla et al., 2018b). Importantly, small differences are found in CRE concentrations between plasma and ISF in healthy patients. Indeed, individual ISF concentrations are strongly correlated with plasma CRE (Pearson´s correlation coefficient of 0.94) (Ebah, 2012), which was additionally confirmed in the case of rabbits (Kayashima et al., 1992). However, it seems that dialysis treatment could increase this difference (Ebah, 2012). Kost et al. (2000) showed this fact by means of an in vivo comparison in rats after provoking a faster CRE extraction through ultrasound. Unfortunately, as in the case of tears, there is a lack of accessible information regarding CRE in CSF.

3. Modern sensing of creatinine

The very first CRE sensor, apart from the Jaffé method, was reported by Meyerhoff's group in 1976 and it consisted of a gas-sensing ammonia electrode (Meyerhoff and Rechnitz, 1976). The electrode partially used the natural monoenzymatic pathway by which CRE is hydrolysed with ammonium generation:

$$A.\,1: Creatinine + H_2O \rightarrow N-methyl-hydantoine + NH_4^+$$

$$A.\,2: NH_4^+ + \alpha\text{-}oxoglutarate + NAD(P)H \rightarrow Glutamate + NAD(P) + H^+$$

The required enzymes are creatinine deaminase (CD, EC 3.5.4.21) (Step A.1) and glutamate dehydrogenase (GDH, EC 1.4.1.3) (Step A.2).

This sensor has served as source of inspiration for subsequent studies based on the indirect detection of CRE by ammonium sensing through various methods (Magalhães and Machado, 2002; Mascini and Palleschi, 1982). In addition, pH detection (Zhybak et al., 2016) and the electrochemical monitoring of the oxidation of NADH were employed for indirect CRE detection on the basis of this reaction (Fossati et al., 1994). Indeed, there has been a general trend over the years to derivatize CRE to another compound measurable primarily through colorimetry or electrochemistry (Killard and Smyth, 2000; Lad et al., 2008; Mohabbati-Kalejahi et al., 2012; Pundir et al., 2013; Randviir et al., 2013).

Table 2 lists papers focusing on CRE detection that have been published since 2014 to date. A preliminary assessment reveals that the use of the Jaffé method is being replaced by other colorimetric approaches as well as electrochemical (bio)sensors. Additional techniques, such as (electro or photo)chemical luminescence (ECL, PL and CL), surface plasma resonance (SPR) and mass spectrometry (MS) have also been proposed in the literature. However, the large and sophisticated instrumentation involved in these approaches is not compatible with POC detection of CRE. Moreover, only colorimetric and voltammetric sensors have been applied in a truly POC manner (three of 33 papers) (Dal Dosso et al., 2017; Kumar et al., 2017; Tseng et al., 2018),

but without achievement of implementation in real scenarios as accurate platforms yet.

The urgent need for decentralizing CRE analytical detection makes it more evident that chromatographic techniques are not appropriate to resolve this analytical challenge despite there being remarkable success in the analysis of the majority of biological fluids at the laboratory scale 15 years ago (Kochansky and Strein, 2000; Walsh and Dempsey, 2002). In principle, the selected technique as a readout for the (bio)sensing of CRE at the POC level will be a compromised situation in terms of the physical implementation of the instrumentation in a portable and easy-to-handle device, rapid data acquisition and interpretation, analytical performance and portability. As continued monitoring of CRE levels is not strictly necessary from a clinical point of view (Kasiske et al., 2001; Schmidt et al., 2017), long-term performance of the sensor is not required, and consequently, disposability of the sensor is a suitable alternative. This is an advantage once encountering possible (bio)fouling effects, which are more dramatic over time (Wang et al., 2018), and recalibration of the sensors to correct response drift (see a deeper discussion in Section 4).

Accordingly, the main analytical features to be considered are: response time, selectivity and linear range. The ideal response time is within seconds-minutes, selectivity should allow for accurate CRE detection in the biological fluid of interest – especially in blood – with a sufficient limit of detection (LOD) and, finally, the linear range must be as large as possible to include both healthy and harmful levels in order to avoid reaching 'false positives' and/or 'false negatives'. Notably, healthy CRE levels in urine, which are in the mM range, are from 10- to 1000-fold higher than in the rest of the biological fluids (Table 1), hence the same analytical technique would not be theoretically applicable to all samples. For one, urine samples are previously diluted, which is not compatible with the POC criteria. As a result, sample selection is also analytically important, apart from attempting to reduce any kind of invasion of the individual during sampling.

Fig. 2 shows the percentage distributions of both the type of samples and methodologies used in the publications listed in Table 2. As observed, serum, plasma and urine are the only samples utilized for CRE analysis. Worth noting is that in the majority of cases, a previous sample dilution is required to be bracketed in the working range of the analytical methodology. Besides, the analysis of just artificial samples certainly appears with a high percentage at ca. 20% (Fig. 2a). Undiluted whole blood and CSF are only utilized in two specific papers, likely owing to the matrix complexity as well as the complex and risky CSF extraction. With respect to the techniques, colorimetric and electrochemical methods continue exhibiting a clear dominance, ca. 38% of the total number of papers in both cases (Fig. 2b). The interest in voltammetry (cyclic voltammetry, CV, and differential pulse voltammetry, DPV) and amperometry has been growing over the years while only one potentiometric sensor has been published so far between 2014 and 2018. In the following, we critically describe the advantages and disadvantages of these techniques.

3.1. Colorimetric detection

In view of improving colorimetric sensing of CRE, some authors have recently reported variations of the Jaffé method (Table 2). Parmar et al. (2016) described a new approach using silver nanoparticles (AgNPs) coated with picric acid to generate the red chromogen. The combination of the NPs dispersion together with a centrifugation step increases the efficiency of the reaction, which leads to a higher selectivity. Despite this approach being successfully applied to detect CRE in diluted CSF and serum, it seems not to be the best option for a POC implementation as these two biological fluids require a rather invasive extraction and matrix separation, respectively, apart from the centrifugation requirement. Later on, Alula et al. (2018) covered AgNPs with citrate to promote alkaline CRE aggregation, demonstrating the possibility of CRE analysis in diluted urine. The main disadvantage is

that the physiological pH of the sample must be modified and maintained at pH 12 to force the aggregation.

Du et al. (2016) proposed a AuNPs-based sensor that takes advantage of the synergistic coordination of adenosine and CRE in the presence of silver ions. Despite measurements in artificial urine and bovine serum being successfully carried out, the validation of the sensor was somehow missed. In addition, He et al. (2015) also made use of a sensing concept based on AuNPs, but the detection principle consists of a direct cross-linking reaction between the CRE and AuNPs, which causes an aggregation that produces an improved change of colour. Despite the advantage of suppressing the use of picric acid, urine samples were diluted 100-fold because of the very low working range at the nM level. Sittiwong and Unob (2015) proposed a similar urinary CRE detection with AuNPs and CRE extraction on silica gel functionalized with sulfonic acid. The aggregation of CRE with AuNPs promotes a colour change that can be observed by the naked eye as well as by spectrophotometry. As in the previous case, the urine samples required a previous dilution step before performing the measurements. Unfortunately, none of these examples shows values related to the accuracy of the sensors against standard methodologies, although the time required to perform the assays seems promising in most of them (between 1 and 10 min).

A slightly different proof-of-concept was reported by Du and coworkers (Du et al., 2015). In this case, the study was based on the synergistic coordination chemistry of CRE and uric acid in the presence of Hg^{2+} on AuNPs' surfaces. Therefore, the colour of the solution changes from red to blue with rising CRE concentrations. The authors claimed the possibility of incorporating a smartphone as an on-field detector and also for on-line data analysis. This work shows recoveries close to 100% (94–103%) for spiked CRE with an estimated analysis time of 5 min. In this regard, the POC potential of this approach may be highlighted when overcoming the sample dilution requirement.

Debus et al. (2015) reported certain technical innovations to develop the Jaffé reaction in an improved setup close to the POC concept. Custom-made platforms were used for this purpose based on circular dichroism spectroscopes and a computer screen photo-assisted technique. CRE analysis in diluted urine needs between 5 and 15 min and demonstrated accuracy deviations of ca. 10% (correlation of R^2 = 0.930 – 0.977 with the Jaffé method), which falls inside traditional clinical tolerance limits. It is noteworthy that, as stated earlier, validations employing the Jaffé method as gold standard and usually display differences at this level (Walsh and Dempsey, 2002). Along the same lines, Fu and co-workers recently implemented the Jaffé reaction in a paper-based platform (microfluidic paper-based analytical device, µPAD) pursuing POC integration (Fu et al., 2018; Tseng et al., 2018). The analytical device serves as a CRE detector for blood and features a reaction zone, which is placed after the serum separation from the blood sample with picric acid and NaOH. When the blood diffuses through the paper-based platform, the serum is separated in the fluidic channel in a capillary manner (Yetisen et al., 2013). The µPAD is, in turn, inserted in a portable detection box that comprises a temperature controller, power source, light source, CMOS camera (active pixel sensors in complementary metal-oxide-semiconductor) and smartphone (connected to the system by USB or WiFi) to collect data in order to build up the POC device (Fig. 3a). The linear range achieved with the colorimetric detection in this device is wide enough to distinguish between healthy and harmful concentrations of CRE in blood/serum. These sensors required between 5 and 8 min to complete the assay and showed a good correlation with the gold standard technique (R^2 = 0.992 – 0.994). Importantly, this is one of the two (bio)sensors that have been truly applied for POC detection of CRE, in this case within whole undiluted blood.

Another approach based on the µPAD configuration in combination with the multienzyme cascade reaction (tri-enzyme pathway), in which CRE is involved, was reported by Talalak et al. (2015):
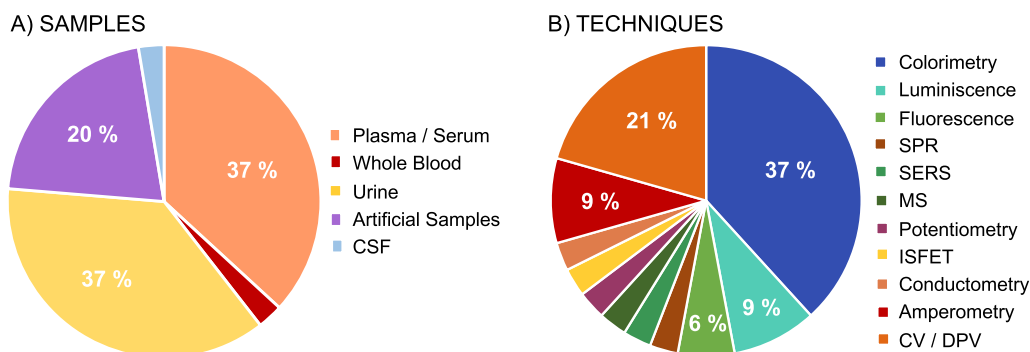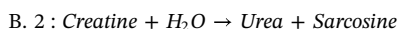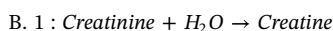
Fig. 2. Summary of the biomatrices and analytical techniques used in modern CRE (bio)sensing. a) Distribution of the samples analysed in the papers listed in Table 2. b) Distribution of the methodologies for CRE detection collected in Table 2.

B. 1 : *Creatinine + H_2O → Creatine*

B. 2 : *Creatine + H_2O → Urea + Sarcosine*

B. 3 : *Sarcosine + H_2O → Formaldehyde + Glycine + H_2O_2*

The enzymes involved in this cascade are creatinine or creatinine amidohydrolase (CA, 1 EC 3.5.2.10) (Step B.1), creatinase or creatine amidinohydrolase (CI, EC 3.5.3.3) (Step B.2) and sarcosine oxidase (SO, EC 1.5.3.1) (Step B.3). It is worth noting that other papers seen in Table 2 also described monitoring the formed H_2O_2 colorimetrically (using a side reaction to generate a coloured complex) (Dal Dosso et al., 2017) as well as amperometrically (Kumar et al., 2017a). Importantly, the other intermediate products could be used for indirect CRE detection. The sensor reported by Talalak et al. (2015) was a paper-based strip with different zones created to first deliver the enzymes and then the reagents necessary to derivatize the generated H_2O_2 to quinonimine for the colorimetric readout. The reported working range allows for CRE detection only in urine with analysis time close to 10 min. Advantageously, the sensor reported by Dal Dosso et al. (2017) based on the same multienzyme cascade reaction was applied in undiluted plasma analysis, being the proposed device was really close to true POC operation (Fig. 3b). The system integrates a cartridge based on a self-powered imbibing microfluidic pump by liquid encapsulation (SIMPLE). This cartridge is composed by different polymeric layers to build the sampling and detection zones. In addition, the bioassay requirements are fulfilled owing to the filtration (and selection of the proper wavelength at 570 nm to follow the colour of the compound generated from the H_2O_2 reaction) of the light provided by six green LEDs for the colorimetric readout. Of benefit, the use of this device permits a reduction in the LOD (~60 μM), and therefore, it is applicable to plasma characterization and could be further applied to diluted urine.

Other reactions have also been used to generate a colourful compound with CRE. Ellairaja et al. (2018) documented an approach where a chalcone, i.e., (*E*)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (PTP), was used as a chemodosimeter to interact with and selectively recognize CRE in serum. The PTP chalcone works both as an absorbance and fluorescence probe. However, it presents a strong pH influence, i.e., at low pH values, the probe displayed two major absorption peaks (at 297 and 407 nm) that vary when increasing the concentration of CRE (measurable with UV–Vis spectroscopy). While this also takes place in fluorescence mode, the PTP probe exhibits an improved stability (lifetime of five weeks). The time necessary to complete the optical assay using is 10 min with recoveries of 88 – 105% for spiked CRE. The main advantage with this sensor is the surprisingly wide detection range (from 1 nM to 1.4 mM), whereas the need for pH control is the main drawback. Although the sensor was applied exclusively to CRE detection in serum, it could be suitable for urine when taking into account this range.

With another approach, liquid crystals (LCs) were utilized for indirect CRE detection (Verma et al., 2016). The concept is based on the principle whereby the surface of the LCs is doped with hexylbiphenyl-4-carboxylic acid (HBA) and a local pH change induces a drastic colour
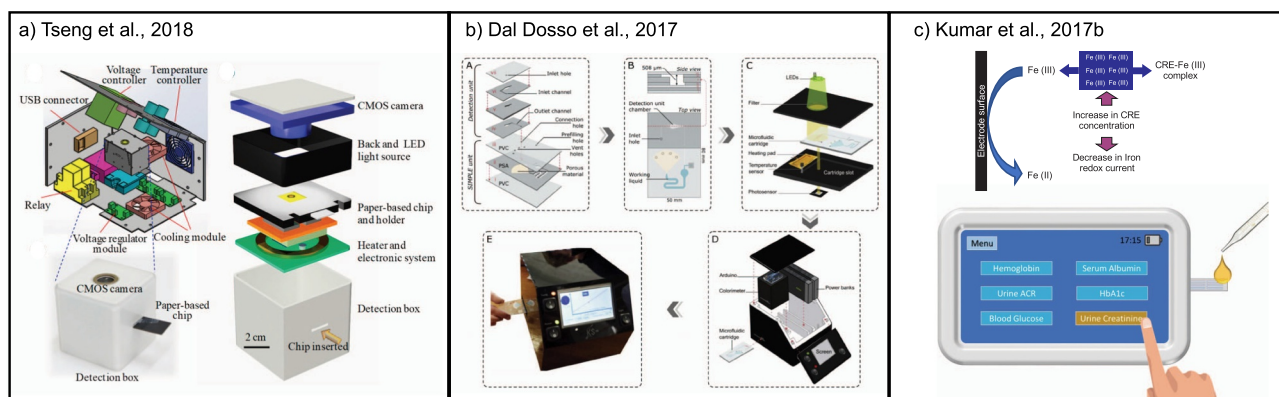

Fig. 3. POC devices for CRE detection reported over the last five years. a) Tseng et al. (2018): Photograph and illustrations of main components of the smart detection device, showing in detail the composition of the detection box. Reproduced from (Tseng et al., 2018), *Copyright (2018), with permission of Elsevier.* b) Dal Dosso et al. (2017): Overview of the Creacard (microfluidic cartridge), colorimeter and integrated Creasensor. A) Fabrication of the Creacard comprising the SIMPLE unit and detection unit. The assembly sequence of the cartridge is presented with red arrows and numbers. B) Top view of the assembled and prefilled Creacard and side view of the detection unit (chamber height = 508 μm). C) Expanded view of the main components of the colorimeter. D) Expanded view of the Creasensor with the colorimeter, an Arduino microcontroller, power banks and screen. E) Photograph of the final Creasensor device in use. Reproduced from (Dal Dosso et al., 2017), *Copyright (2017), with permission of Elsevier.* c) Schematic representation summarizing the work presented by Kumar et al. (2017b): Mechanism of Fe(II)-based electrochemical detection of CRE and POC device in test mode with the inserted disposable test strip in which the sample is deposited.

change in LCs from a bright to dark optical appearance. In the sensor, the local pH change occurs owing to the generation of NH_4^+ ions that result after enzymatic conversion of CRE. The system was only applied to detecting CRE levels in artificial samples up to 50 μM (healthy levels), but also at harmful levels (synthetic samples of 100, 150 and 1000 μM CRE). The orientational ordering of the LCs was determined with polarizing optical microscopy (×50). The system may have potential for further POC application if the optical microscopes are replaced by a simpler colorimetric detector, such as a camera. Nevertheless, the demonstration of the suitability for real sample analysis is mandatory before this implementation.

Importantly, before the selected period that this review covers, some authors investigated CRE recognition by hydrogen binding with distinct neutral hosts and supramolecular receptors with possible UV recognition of the formed complex (Buhlmann and Simon, 1993; Buhlmann et al., 1998). However, none of them further evolved because of the better performance of the Jaffé method.

3.2. Electrochemical techniques

Voltammetric detection of CRE employing electrodes based on molecularly imprinted polymers (MIPs) was reported in three recent papers (Diouf et al., 2017; Rao et al., 2017; Wen et al., 2014). In principle, the cavity developed in the MIPs has the specific size and shape to selectively host the analyte of interest. Diouf et al. (2017) reported a MIP-based sensor where the binding sites for CRE are generated onto a carboxylic polyvinyl chloride layer firstly deposited on gold electrodes. This sensor selectively identifies CRE with a LOD at the nM level (0.14 nM) and was successfully applied to detect CRE in diluted urine. While the reported low LOD would allow for CRE detection in any biological fluid subsequent to dilution, there was a lack of interference. This may limit application to whole blood or other samples. Besides this, the long analysis time required for the entire assay (ca. 2 – 5 h) is a marked disadvantage.

The studies presented by Rao and Wen showed two similar examples of MIPs based on the use of NPs, such as NiNPs or Fe_3O_4NPs, together with polyaniline (PANI) (Rao et al., 2017; Wen et al., 2014). In both cases, glassy carbon electrodes are modified with the MIP and nanoparticle-PANI composite by electropolymerization, yielding similar LODs (0.2 and 0.35 nM respectively). The sensors were applied to detect CRE in spiked (artificial) samples, leading to recovery percentages close to 100%. In addition, one of the main disadvantages of using voltammetric sensors based on MIPs is the great amount of time (ca. 1–3 h) needed for preparation taking into account the washing, incubation and polymerization steps.

Other voltammetric sensors are based on the monitoring of CRE following selective binding with Cu(II), Fe(III) or quantum dots (QDs) (Raveendran et al., 2017; Kumar et al., 2017b; Hooshmand and Es'haghi, 2017) as well as enzymatic conversion to cysteine (Desai et al., 2018). Raveendran et al. (2017) proposed a sensor capable of detecting CRE by voltammetry, in particular monitoring the activity of a copper-CRE complex. The sensor showed a wide linear range from 6 to 378 μM (LOD = 0.07 μM) that allows distinguishing between healthy and harmful concentrations as a result of CRE analysis in both serum and diluted urine (recoveries 95 – 98% of spiked CRE). Nonetheless, the high selectivity of this sensor for CRE in serum was brought about by a pre-incubation step of the sample with PbO_2 powder, which ensured the rejection of interferences, such as uric acid during the detection. Note that all these steps made the approach incompatible with the POC concept, apart from the really long analysis time (more than 30 min).

The sensor presented by Kumar et al. (2017b) was based on a carbon-printed electrode coated with a $FeCl_3$ cotton fibre membrane with the ability to measure CRE in urine samples without previous dilution (Fig. 3c). Remarkably, this is the only voltammetry sensor that displayed major success as a POC CRE detector. Detection was based on CRE forming complexes with metals, such as platinum, copper, iron or

palladium (Lad et al., 2008). Essentially, after the addition of CRE, part of the free Fe(III) binds CRE, thereby forming a stable complex. This binding produces a decrease in available Fe(III), which is observable in terms of the reduction of the peak current observed at the voltammetric wave. This POC device does not require any pre-treatment of the sample and provides a CRE concentration in about one minute. Another advantage is that $FeCl_3$ chemistry has no interference via albumin, and this is very useful regarding the albumin-to-CRE ratio detection. However, the current limitation of this sensor is in relation to the linear range that only includes healthy CRE levels. Moreover, the authors claimed further steps based on sensor evaluation regarding the long-term stability and reliability of the test strips as well as interference studies with samples from patients at advanced stages of renal disease (macroalbuminuria).

In other work, QDs were utilized for selective CRE recognition (Hooshmand and Es'haghi, 2017). However, the sensitivity of this approach was limited by the amount of QDs, sample pH, equilibration time of the sample with the QDs (0, 10 or 15 s) and scan rate in the voltammetry readout. Regarding the latter, low scan rates are preferable (0.01 and 0.05 V s^{-1}). The sensor displayed a wide working range, which permits CRE detection in urine and serum. In our opinion, the translation of this concept into a paper-based platform able to separate plasma from blood *prior* to the QD-based detection could be considered a further step towards a potential POC device.

Desai et al. (2018) demonstrated the indirect detection of CRE in spiked urine samples by CV and DPV analysis of cysteine. For this purpose, the cysteine protease enzyme, papain, was immobilized in a screen-printed electrode modified with carboxyl-functionalized multi-walled carbon nanotubes. The amino group of papain is, in turn, covalently bound to the carboxyl group on the electrode surface. Then, the interaction between papain and Cystatin C is electrochemically monitored, showing an extremely low LOD of 0.006 nM with a minimal requirement of sample volume (6 μL). The main disadvantage is that the presented working range means any sort of sample has to be diluted prior to analysis, thus inducing more error in the CRE detection and being somehow incompatible with the POC concept.

Dasgupta et al. (2018) reported indirect CRE detection by amperometric monitoring of Fe(III) concentration after its reaction with CRE, a similar concept to that reported by Kumar et al. (2017b), but the interaction between CRE and $FeCl_3$ occurs directly in solution, which takes analysis time of 10 min. Despite an appropriate working range being presented, the methodology should to be heavily evaluated considering real serum samples and interference studies. The other two papers with amperometry readout comprise enzymatic reactions where CRE is involved (see Table 2) displaying faster response times (few seconds). Zhybak et al. (2016) immobilized the required enzymes by drop-casting a solution of CRE deiminase and urease enzymes onto a Cu electrode modified with PANI and Nafion. Conversely, Kumar et al. (2017a) employed an immobilization procedure comprising the dip coating of the electrode inside a solution that contains NPs of creatininase, creatinase and sarcosine oxidase enzymes. The NPs aggregate and attach onto the electrode surface after 2 h. In both cases, analytical performance is apparently promising with respect to POC CRE detection (response time of 15 and 2 s, LOD of 0.5 and 0.01 μM, and robust storage stability of three months without losing analytical performance as well as eight months with a loss of only 10% of initial enzymatic activity in Zhybak and Kumar studies, respectively). Indeed, these biosensors have been successfully applied for CRE detection in (diluted) serum. Both sensors exhibited a robust correlation compared with the standard spectrophotometric method during their validation.

Another example based on electrochemical detection comprises an ion-sensitive field effect transistor (ISFET) in Ozansoy et al. (2017). In this investigation, the immobilization of the CD enzyme was accomplished on zeolite-based electrodes using glutaraldehyde. For this purpose, the surface of the sensor was firstly modified by drop-coating four different zeolites together with the enzyme. These zeolite-based sensors

all displayed improved analytical parameters for CRE detection with sensitivities between 21 and 30 μA/mM, LOD of 5 μM and a response time of 120 s. The sensors were only applied to CRE detection in artificial samples.

CRE detection has been additionally accomplished by exceptionally simple electrochemical techniques, such as potentiometry and conductimetry. Indeed, from the ammonium detection introduced by Meyerhoff and Rechnitz (1976), other authors have described potentiometric biosensors with enhanced analytical performances, including the integration of a chitosan membrane with the immobilized enzyme and implementation of the ammonium-selective electrode in flow-injection analysis (Radomska et al., 2004a; Magalhaes and Machado, 2002). The conversion of CRE into a cation that is measurable potentiometrically was always necessary based on the absence of an effective receptor (known as an ionophore in the jargon) to directly sense CRE (or its cation, creatininium, at pH values below 3.8).

To this end, Ballester and colleagues reported an effective ionophore-based potentiometric sensor for creatininium (Guinovart et al., 2016, 2017), a calix[4]pyrrole-based molecule that offers a suitable selectivity for creatininium considering the main interfering ions in biological fluids: $logK_{urea,CRE} = -4.3$; $logK_{Ca,CRE} = -4.8$; $logK_{Na,CRE} = -3.7$; $logK_{K,CRE} = -2.5$; $logK_{NH4,CRE} = -2.3$; $logK_{creatine,CRE} = -3.5$. Despite the potentiometric sensor claimed to be an all-solid-state type, in fact, it belongs to the well-known coated wire electrode (CWE) underscored by Cattrall et al. (1974). This is in part because the assembled sensor does not incorporate any ion-to-electron transducer, which undoubtedly negatively impacts stability and drift of the potentiometric response. However, the authors reported adequate analytical performance, such as wide linear range embracing healthy and harmful CRE levels in diluted urine and plasma. In addition, the sensor was satisfactorily validated with the gold standard Jaffé method. The main disadvantages are the need for buffering the sample at pH 3.8 to transform all the neutral CRE into the creatininium cation together with the mandatory necessity of diluting the sample by factors of 100 and 10 for urine and plasma, respectively, to minimize biofouling contributions to the electrode response. Despite these drawbacks keeping this sensor away from POC application, it seemingly demonstrated potentially favourable results detecting CRE in diluted samples from both patients that are healthy and those suffering from renal dysfunction (Guinovart et al., 2016).

Finally, Braiek et al. (2018) presented conductometric detection of CRE by means of the immobilization of the CD enzyme onto a composite film (composed of polyvinylalcohol/polyethyleneimine/gold nanoparticles, PVA/PEI/AuNPs). The final conductometric biosensor showed robust reproducibility along with broad linear range and was used for CRE detection in spiked artificial serum samples (estimated time of 3 min). Validation with real samples and correlation via standard methods were not addressed in this study.

3.3. Other methods

CRE detection based on fluorescence was reported in two different works by the groups of Ellairaja (already discussed in colorimetric methods) and Pal (Ellairaja et al., 2018; Pal et al., 2016). In the latter work, a Pd^{2+}-naphthalimide-based florescence light-up probe (FCP-Pd) was developed to detect CRE in serum samples as low as 30 μM. The detection was based on the fluorescence recovery of FCP by removing Pd^{2+} (the well-known heavy atom-quenching effect) from the FCP-Pd complex after an incubation step of 30 min in the presence of CRE. During this time, CRE reacts with Pd^{2+} to form a complex (Pd$(CRE)_2Cl_2$), therefore providing free FCP ligand. The approach was validated with the colorimetric Jaffé method, showing recoveries of 95–99% for spiked CRE, therefore indicating the suitability of this new system for further clinical applications.

Luminescence approaches were also reported, including chemiluminescence (CL) (Hanif et al., 2016), photoluminescence (PL) (Babu

et al., 2018) and electrochemiluminescence (ECL) (Babamiri et al., 2018), the latter displaying remarkably broad working ranges. Hanif et al. presented enhanced CL measurement of the reaction between CRE and hydrogen peroxide in the presence of cobalt ions. The CRE determination in diluted urine samples was accomplished by coupling CL detection to flow-injection analysis, which involves a really sort analysis time of some minutes. Despite the technique's simplicity, the pH influence is relatively marked (i.e., CL intensity rises with higher pH because it facilitates the deprotonation of H_2O_2 and oxidation of CRE as well as the solubility of cobalt ions decreasing in the medium).

Babu et al. (2018) reported a PL approach based on carbon nanodots (CNDs) on carbon-gold nanocomposites (C-Au NCs) functionalized with bovine serum albumin (BSA). The intensity of PL diminishes upon increasing concentration of CRE because of the removal of BSA from the composite after reaction with CRE. The sensor displays an extremely broad working range (17 μM–1.7 M) where healthy and harmful levels of CRE in all undiluted biological fluids are included. The work also provided a deep biocompatibility study producing promising results in comparison with other fluorescent dyes and QDs. However, there is a lack of demonstration of the sensor in real samples. Babamiri et al. (2018) reported the determination of CRE using ITO-MIPs electrodes modified with nickel nanoclusters (NiNCs) as ECL emitters. The ECL signal is produced when the electrode reacts with tri-*n*-propylamine after CRE insertion in the corresponding MIP cavities, which is a process that takes a longer time (15 – 30 min) than other concepts for CRE recognition. Unfortunately, the results were validated by spiking the samples with known CRE amounts and calculating recoveries, which is not the most convenient validation protocol.

Stamping surface-enhanced Raman scattering (S-SERS) was recently utilized for CRE detection in artificial samples (Li et al., 2015). The study showed the combination of the S-SERS technique with nanoporous gold disk (NPGD) plasmonic substrates. It was shown that the CRE peak intensity rises as a function of CRE concentration owing to the interaction with the NPs. S-SERS was claimed here as an excellent method to obtain a CRE spectrum at 100 nM concentration. In addition, a study based on surface plasmon resonance (SPR) was recently published (Pothipor et al., 2017) where the transmission surface plasmon resonance-imaging (TSPR-i) was investigated on a gold substrate, which was modified with AgNPs by means of a microfluidic cell. The TSPR-i intensity first decreased after the deposition of the AgNPs and then was raised after CRE incubation for 1 h because the strong CRE-AgNPs interaction. The displayed working range at the mM levels restricts the application of this principle to urine, but, unfortunately, the authors of both works only provided evidence for CRE detection in artificial samples (see Table 2).

Solid phase microextraction (SPME) coupled to ultrafast evaporation with ion mobility spectrometry (IMS) readout has been proposed by Jafari et al. (2015). The approach was validated with the Jaffé method in two serum and urine samples showing recovery values close to 100%. Both samples required a 1:1 dilution for the analysis in order to fit within the working range. The very low sample volume required for the analysis (10 μL) can be considered a remarkable advantage. Nevertheless, in order to be more consistent with respect to reliability of this new approach, validation with a higher number of real samples from healthy and CKD patients should be performed additionally, as put forth by the authors.

4. General criticism of the most promising principles for creatinine (bio)sensing at the point-of-care level

Having described all the techniques reported for CRE detection over the last five years, the following conclusions can be extracted considering POC application of all these (bio)sensors:

(i) Depending on the working range of the (bio)sensor, this will be suitable for various kinds of biological fluids. Considering urine, a

range from 1 to 30 mM will allow for the identification of healthy and harmful CRE levels, while in the case of blood (or plasma/serum), this range is from 10 to more than 150 μM (see Table 1). The latter would be also suitable for the remaining biological fluids. Of note, these estimated ranges would permit one to precisely quantify CRE levels at healthy levels and qualitatively identify outlier cases.

(ii) While working ranges at the mM level restrict the use of the (bio)sensors for urine, μM levels permit CRE detection in blood and diluted urine. However, in the specific cases where nM levels or even lower are achieved, dilution is required for all samples (Du et al., 2015; Desai et al., 2018; Rao et al., 2017; Diouf et al., 2017). Unfortunately, dilution steps impede the POC application of the (bio)sensor.

(iii) Detection methods that do not imply colorimetric or electrochemical readouts generally involve more sophisticated sensing principles and larger instrumentation. Within this context, fluorescence and luminescence probes seem to be more suitable for POC CRE detection in the near future (Ellairaja et al., 2018; Pal et al., 2016; Babamiri et al., 2018; Babu et al., 2018; Hanif et al., 2016). However, data interpretation is also more complex than with colorimetric and electrochemical options considering that the data will be handled and interpreted by non-expert end users. In our opinion, this is the reason why colorimetric and electrochemical CRE detection is more spread.

(iv) Apart from the attempts to translating the Jaffé method at the POC level (Fu et al., 2018; Tseng et al., 2018), only one colorimetric sensor and other voltammetric were successfully applied for this endeavour (Kumar et al., 2017b; Dal Dosso et al., 2017; see Fig. 3). Significantly, the only path to establish the traditional Jaffé reaction for CRE POC detection in blood is using a paper-based platform that allows for the in situ separation of plasma from the blood sample in the same device without the need to transport the sample to a centralized laboratory. Herein, it is absolutely necessary to investigate how the physical separation process itself specifically influences CRE as a neutral biomolecule.

The biosensor reported by Dal Dosso et al. (2017) is based on the CRE enzymatic reaction to produce H_2O_2, which is later derived into a coloured compound. The monitoring of one of the possible products when CRE is involved in enzymatic reactions is a widely used strategy for CRE detection in both blood and urine (see Table 2). Indeed, the majority of the commercially available devices claiming POC CRE detection are based on an enzymatic reaction coupled to amperometric or spectrophotometric detection (Shephard, 2011). Despite these devices being suitable for use in clinical laboratories after sample collection, none of them really fulfil the current requirements for a POC system (Mahato et al., 2017; St John and Price, 2014), mainly because they are cumbersome to measure next to the patient. Importantly, there are two devices (called as iSTAT and Stat Sensor)[4] with true POC potential in terms of dimensions, blood detection, analysis time (2 and 0.5 min, respectively), sample volume (65 and 1 μL, respectively) and working range, including healthy and harmful levels (18–1768 μM and 27 − 1056 μM, respectively), but there are still improvements necessary regarding selectivity. Other examples of commercial devices are Roche-Reflotron Plus and Abaxis-Piccolo that offer excellent analytical features in terms of analysis time (3 and 8.5 min, respectively), sample volume (30 and 9 μL, respectively) and working range (45–884 μM and 18–1768 μM, respectively), but they involve the use of large and heavy instrumentation (5 kg in both cases), which limits its use as POC devices.

(v) Among all the sensors collected in this review, only 7 of the 33

papers can be considered truly biosensors, i.e. the sensor uses a recognition element of biological nature (Perumal and Hashim, 2014), and only the colorimetric sensor developed by Dal Dosso et al. has been applied for POC CRE detection. In this context, CRE detection is achieved through an enzymatic reaction and the required enzyme(s) are traditionally immobilized in any of the parts of the device. Advantageously, immobilized enzymes are more resistant to external changes and permits to develop the enzymatic reaction in a controlled and confined environment. Indeed, CRE biosensors collected in Table 2 present long-lasting lifetime as well as the faster analysis times (between seconds and few minutes).

Apart from dip-coating and drop-casting approaches reported by Kumar et al. (2017a) and Talalak et al. (2015), there is a very broad catalogue of strategies for general enzyme immobilization (Cosnier, 1999; Datta et al., 2013) that may be used in further designs for POC CRE detection (i.e., improving lifetime, enzyme activity and, indeed, putting forth physical/chemical barriers to interference (Yan et al., 2011)). While, all these techniques are in principle easy to be implemented with any type of enzyme, the complexity of the procedure increases in the case of cascade reactions involved in the biosensing process. Moreover, it is absolutely necessary to assure the correct performance of all the enzymes under optimal pH and temperature, which sometimes limits the use of the biosensor or involves a change in the pH of the sample.

Apart from an increase in the cost of the sensor manufacturing, the involvement of multiple enzymatic reactions additionally increases the time required for the analysis, which will be here based on the needed time for the sample to reach the reagents and compounds in the device, the reaction times associated with the enzymatic reaction and the generation of coloured products (if necessary) together with the response time of the biosensor. For instance, in the case of the tri-enzyme reaction, this time is close to 5 or 7 min depending on the approach employed to detect H_2O_2 (Kumar et al., 2017a; Dal Dosso et al., 2017). Beneficially, other reactions based on synthetic enzymes may be also explored in the close future seeking to overcome all drawbacks of current biosensors.

Other advantage involved in the use of biosensors for CRE detection is the possibility of regeneration of the analytical signal. Although the disposability of the sensor undoubtedly fits with the POC concept, if its cost does not permit this way of operativity, regeneration of the sensing part is the best option. The selected process for this purpose may differ according to the nature of the sensing/recognition element. According to Goode et al., in general, there are five types of mechanisms for the regeneration of biosensors: (i) chemical regeneration to alter the solvent environment and favour the unbinding of the target; (ii) thermal regeneration that gives molecules an increased kinetic energy allowing binding forces; (iii) regeneration promoted by enthalpic interactions (mainly related to polar or ionic charges and potential energy involved in chemical bonds); (iv) through entropic interactions (in case of hydrophobic properties), and finally, (v) electrochemical regeneration by applying negative potentials that provide highly controlled and localized processes (Goode et al., 2015).

(vi) The device reported by Kumar et al. (2017b) may awaken interest of the reader for different reasons (Fig. 3c). First, it is not a biosensor per se because it does not comprise the use of any enzyme (biological component of the biosensor). Instead, it is based on the CRE interaction with Fe(III). Second, it is the only POC device that measures CRE in a fast, reliable and disposable manner in urine. Last but not least, the sensing principle is very simple, and the observed signal is easy to interpret with results that can be validated. It is worth highlighting that according to Table 2, there is an emerging trend to utilize reactions involving CRE rather than enzymatic ones. Importantly, there are many different reactions that have been explored, but in our opinion, efforts to show the true

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[4] https://www.pointofcare.abbott/us/en/offerings/istat/istat-test-cartridges; http://www.novabio.us/statstrip-creatinine/

potential for POC CRE detection is still necessary, for example, binding with adenosine, citrate, chalcone or AuNPs in the presence of NPs (Du et al., 2016; Alula et al., 2018; Ellairaja et al., 2018; He et al., 2015) or with Pd (Pal et al., 2016), H_2O_2 (Hanif et al., 2016), Cu(II) (Raveendran et al., 2017) and QDs (Hooshmand and Es'haghi, 2017) among others.

In general, any POC detection involves the following analytical requirements for the sensing principle (Junker et al., 2010; Mahfouz et al., 2013; Zarei, 2017): fast response time (from seconds to minutes); reliability (clinical tolerance limits generally require 10 – 15% accuracy in the validation protocol); adequate selectivity to afford the clinically demanded working range and LOD; no or minimum risk of sample contamination (which is incompatible with any sample manual handling, such as dilution, plasma extraction in blood, mixture of reagents and others); disposability to avoid any risk of cross-contamination; easy or null calibration protocol; preparation of the sensing principle must be compatible with mass production and, in the case of requirements, only easy steps should be carried out by the end-user because the reliability of the quantification will be influenced by all these steps; tolerable provision of 'false positives' and 'false negatives'; among others.

Considering the three (bio)sensors for CRE detection found in publications of the last five years and of which the authors remark they developed POC devices, it is certain they are not. None of them really fulfil all the POC requirements, although they are very close (Dal Dosso et al., 2017; Kumar et al., 2017b; Tseng et al., 2018). The amperometric biosensor developed by Kumar et al. (2017b) is based on the interaction between Fe(III) immobilized on a strip and CRE in the sample. It presents a LOD in the mM range and it is therefore applicable only to urine analysis without the need for any pre-treatment. The device is already implemented in a friendly interface as to how the disposable strip is placed, and in just one min, the CRE content in 300 μL of urine is obtained (Fig. 3c). The device was validated in a first step with 50 urine samples and the results were well-correlated with those obtained through the Jaffé method. There is no temperature influence in the range of 20–40 °C, and no interference from albumin, though other interferences were not tested. Regrettably, while the calibration graph is considered a universal one and therefore implemented into the interface and software to calculate CRE concentration, there is no exhaustive investigation of the appropriateness of this standardization with a statistical approach. It would be necessary to indicate the limitation in accuracy and precision of the quantitative detection as well as establishing the re-calibration frequency of the sensor. Indeed, fundamentally speaking, amperometric sensors always need re-calibration except in the case that linearity is found between the analyte concentration and the involved charge (i.e. coulometric sensors; Cuartero et al., 2015).

In addition, the preparation of the strip necessitates several steps - deposition of $FeCl_3$ solution in cotton substrate that is then placed on the plastic strip with the PINE electrode and dried at 34 °C in an oven. Furthermore, there is no specification surrounding the stability of the electrodes, i.e., if this is an approach compatible with mass production or if some of the preparation steps need to be executed by the end user. It could also be necessary to further demonstrate the utility of the sensor for the identification of harmful clinical situations. Despite these uncertainties, it is clear that the device proposed by Kumar et al. (2017b) is very close to POC CRE detection (Fig. 3c). In a very recent work, the authors presented the application of the POC device for early detection of diabetes kidney disease (DKD) (Kumar et al., 2017b). The device is feasible to quantitatively measure five different biomarkers related to DKD and Diabetes mellitus among which urine CRE and the ACR ratio are presented. This study represents a major step forward in realizing a robust and scalable POC multianalyte sensor able to generate clinically relevant observations in less than one minute. The validation of this patented device was accomplished using more than 500 samples from 400 patients suffering DKD at stage 2 and 30 patients at

stage 3, showing acceptable correlation with the Jaffé method (0.85 – 0.95) (Kumar et al., 2018). The disposability nature of the sensors based on traditional strips facilities the use in such large number of samples.

The colorimetric biosensor reported by Dal Dosso et al. (2017) is not truly a POC device because it is not able to measure directly in blood as serum isolation is mandatory. As a consequence, although the authors stated that this is a POC device (Fig. 3b), we would like to put forth we believe it is a close-to-POC device. Through further steps, the authors may resolve the issue of application in whole blood using a paper-based device. Furthermore, a more exhaustive investigation of the standardization of the calibration graph, the lifetime of the sensor (and enzymes) as well as a more elaborated validation with a higher number of real samples will be necessary in the near future while also identifying harmful clinical situations. In addition, the fabrication and handling of the device seems to be complex (Fig. 3b). Interestingly, considering lifetime studies of biosensors, long-term investigations of the activity of the enzymes were conducted during more than three and eight months (Zhybak et al., 2016; Kumar et al., 2017a). In this regard, some authors claim that the remarkable robustness and stability of immobilized enzymes become from the structural changes produced during the immobilization procedure that generates a new microenvironment (totally different from the bulk solution) where the enzyme acts in a more controlled manner (Homaei et al., 2013).

Lastly, the colorimetric sensor developed by Tseng et al. (2018) still features the majority of the drawbacks innate to the Jaffé reaction (already mentioned in the previous sections), though it resolves blood utilization (Fig. 3a). However, in our opinion, it is necessary to reach a paradigm shift from the Jaffé reaction to another different strategy that provides authentic CRE detection fulfilling all POC requirements. Consequently, we are now in the position to answer the question that was raised in the abstract of the present review: To what extent is creatinine detection already solved in healthcare applications, or is still a challenge? The response is straightforward. While CRE detection is possible at the laboratory scale via the Jaffé method and others (Shephard, 2011), there is not yet one biosensor for true POC detection of CRE that allows for trustworthy clinical decision-making. As there is an urgent need to deal this challenge, novel CRE-sensing concepts have been proposed over the last five years and are positively evolving towards POC CRE detection.

Within this context, the ideal device may probably use one single blood drop (fingerprint technique), or low urine volume (after 24 h collection), or indeed could be conceived as a wearable sensor embedded in a diaper or urine collection bag, or even implemented in a microneedle-based platform for advanced CRE detection in ISF. In these latter cases, additional biocompatibility and toxicology studies related to the skin are necessary for a final prototype.

On the other hand, efforts carried out in the laboratory surrounding the investigation of (bio)sensor performance must be reformulated in order to consider mandatory information for the POC concept that go unnoticed in recent publications. We are referring to: (i) lifetime evaluation of the (bio)sensor (and required materials) to establish suitability for further mass production and easy utilization by non-expert end users; (ii) investigation of any (bio)fouling effect (Wang et al., 2018) during the time in which the sensor is in contact with the sample (especially in the case of blood); (iii) characterization of the disposability feature, standardization of the calibration graph and re-calibration frequency by using one- or two-point protocols, as in the glucometer, pH meter or recently established environmental sensors (Cuartero et al., 2017); (iv) deeper interference study considering matrix effects and avoiding the need for sample dilution; (v) clinical validation based on gold standard techniques (Jaffé method, HPLC or any other commercially available device) and using a significantly higher number of samples (> 50 for prototypes during early-stage development), including healthy and harmful levels; and (vi) technical requirements, such as cost-effective fabrication, self-powered devices, data harvesting and (wireless) connections, need to be additionally

considered, albeit this is outside the scope of the present review.

5. Conclusion

The clinical importance of detecting CRE levels in urine and especially in blood (serum) has been extensively supported by alliances between chemists and clinicians. Modern creatinine (bio)sensing is based on distinct approaches, among them involving the Jaffé reaction, multienzyme cascades or specific creatinine binding. The two common detection methods used are colorimetry (VIS absorption) and electrochemistry (including voltammetry and amperometry) though fluorescence and luminescence detection that have recently showed promising features for creatinine detection. The majority of the works reported over the last five years are applied to urine or plasma/serum, while only one sensor is suitable for undiluted whole blood detection (Tseng et al., 2018) and another demonstrating to be appropriate for cerebral spinal fluid (Parmar et al., 2016). Of all the inspected (bio)sensors, only three are claimed –by the authors– to be for creatinine detection at the POC level (Dal Dosso et al., 2017; Kumar et al., 2017b; Tseng et al., 2018). Nevertheless, we believe that these (bio)sensors need further improvements and much more intensive evaluations towards true POC applications in healthcare. In contrast, clinical decision-making involving CRE levels currently rely on the Jaffé method and, to a lesser extent, on other commercial devices based on the amperometric monitoring of CRE enzymatic reaction products. As a result, the analysis of CRE is yet to be centralized at clinical laboratories, impeding data acquisition in real time and therefore delaying the associated medical action. Certain key facts highlighted herein may contribute to the establishment of the new routes towards a definitive solution for CRE discernment in healthcare applications. Experts in the field have pointed out the associated drawbacks using the Jaffé method and because of that, new sensing principles have been actively proposed. Importantly, other biological fluids with great potential for CRE detection are not yet fully explored. This is the case for saliva and ISF. To date, none of the reported strategies are suitable for the direct detection of CRE in whole blood, and the only device that uses blood for the analysis includes the previous plasma separation by means of capillaries via a paper-based platform. Consequently, it seems that a POC device based on the fingerprint technique is not accessible with modern CRE (bio)sensing. Finally, there are a number of weaknesses in the way that the (bio)sensors are characterized, mainly related to calibration, potential for mass production and appropriateness for identifying healthy and harmful concentrations, that must be addressed in the near future when developing new devices. We wish that the present review provides stimulation and excitement while addressing this important challenge - CRE sensing at the POC level.

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Declaration of interest statement

The author declare that they do no known competing interest or personal relationships that could have appeared to influence the work reported in this paper

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