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Review—Enzymatic Strips for Detection of Serum Total Cholesterol with Point-of-Care Testing (POCT) Devices: Current Status and Future Prospect

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Redundant cholesterol in human blood can cause severe health problems such as heart disease, coronary artery disease, arteriosclerosis, hypertension, cerebral thrombosis, etc Simple and fast cholesterol determination in blood is essential and benefit for early diagnosis. However, the abnormal level of cholesterol requires long-term and sensitive monitoring, which can be time-consuming and laborious for the patients to go to the hospital for the medical examination. To address this issue, the enzymatic strip detection may provide an optimal approach. Combing with the advantages of point-of-care testing (POCT), enzymatic strip detection of serum total cholesterol is continuously being widely used. This review summarizes the research on enzymatic strip detection of serum total cholesterol for POCT by colorimetric and electrochemical method, which may guide further research. It comprises the advantages of POCT, necessity of cholesterol testing, current status using colorimetric and electrochemical method, challenges and future prospect. Considering the current social demand and production issues, the colorimetric method is more likely to achieve mass production. Above all, the enzymatic strip detection of serum total cholesterol for POCT presents promising prospects for the fast cholesterol monitoring and will be further developed after those issues being addressed.
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Point-of-care testing (POCT) is a laboratory-medicine discipline which endeavors to make diagnostic techniques and instruments, which is easily accessible at patient's beds, in outpatient clinics, at sides of accidents or even at patient's home. This concept was brought to the public since the late 20th century and early 21st century.[1] With the growing demands for quick, convenient, affordable on-site diagnostic information, POCT has gained more and more attention both in academia and industry.[2–5] POCT is now becoming one of the most promising fields in the in vitro diagnostic industry and is widely used in clinical testing,[6–10] major epidemic monitoring,[11–14] individual health management, food safety monitoring,[15–17] environmental monitoring,[18,19] on-site law enforcement testing (e.g. traffic, drugs),[20,21] military and disaster relief,[5,22] etc.

Among the numerous POCT applications, cholesterol detection research is in full swing. Cholesterol is a sterol lipid with its structure shown in Fig. 1.[23] It is a derivative of cyclopentane polyphenanthrene.[24] which is widely found in animals, especially in the brain and nerve tissues, and in the kidney, spleen, skin, liver and bile. It is an indispensable and important substance in animal tissue cells. It is not only involved in the formation of cell membranes, but also an important raw material for the synthesis of bile acids, vitamin D, adrenal cortical hormones and sex hormones.[24] Moreover, cholesterol is a crucial component in lipoproteins, which are used to transport hydrophobic molecules (such as fat) in hydrophilic media, like blood.

Normal cholesterol levels vary by nationality. Based on a large number of research data, the guidelines for prevention and treatment of dyslipidemia in Chinese adults was released in May 2007. The stratification standard of total blood cholesterol level in Chinese adults is given: lower than 200 mg dl^{-1} (5.17 mM) is an appropriate range; 200–239 mg dl^{-1} (5.18–6.20 mM) is a marginal increase; and higher than 240 mg dl^{-1} (6.21 mM) is a rise.[25,26] Studies have well demonstrated that elevated total cholesterol is associated with cardiovascular disease, one of the leading causes of death worldwide.[27–32] Beyond that, the abnormal levels of cholesterol or its precursors are related with various human diseases such as depressive disorder,[33] Smith-Lemli Opitz syndrome,[34] non-insulin-dependent diabetes mellitus,[35] acute pancreatitis,[36] brain disease,[37] cystic fibrosis,[38] and even cancers.[39] Interestingly, even though elevated serum total cholesterol levels are associated with increased risk of cardiovascular mortality among the middle-aged adults, it does not happen in the elderly. Avraham Weiss etc used 1852 patients as samples to study the relationship. The data showed that for the very elderly hospitalized subjects, increased levels of serum total cholesterol and albumin may be associated with reduced mortality risk.[40] As cholesterol provides an effective indicator of various diseases, the quick, sensitive and affordable test assays for cholesterol are in great necessity for daily monitoring and/or early diagnosis of cholesterol-related diseases.

On account of the necessity of cholesterol testing, many detection technologies are springing up. The classical chemical methods include Sperry-Webb, Zak-Henly and Abell-Kendall methods. Sperry-Webb method named after Sperry, W. M. and Webb W., who made modification to the original Schoenheimer-Sperry method for cholesterol determination, reported in 1950.[41] In this method, Liebermann-Burchard reagent is often used. Liebermann-Burchard reaction is carried out in acetic acid-sulfuric acid-acetic anhydride medium resulting in blue-green product.[42] Zak-Henly method, based on Zak reaction, is performed in acetic acid-sulfuric acid medium with addition of Fe^{3+} ions, leading to red product.[43,44] These two methods are unfortunately prone to interfere with from hemoglobin and bilirubin. Besides, corrosive reagents are used in these two methods, which is not environmental-friendly. Abell-Kendall method involved sample hydrolysis, cholesterol extraction, and determination of cholesterol levels by spectrophotometry.[45] The main disadvantage of the measurement procedure of Abell-Kendall method is non-specificity to cholesterol, because some interfering compounds such as non-cholesterol sterols, cholesterol precursors, and oxidation products can produce chromophores which are measured at the same wavelength as cholesterol.[46] In conclusion, these methods, though relatively simple and inexpensive to perform, are not often used in POCT due to its complex multi-step procedures. Other non-enzymatic techniques such as gas–liquid chromatography,[47] high performance liquid chromatography (HPLC).[48] and spectrophotometric.[49] have been reported for the

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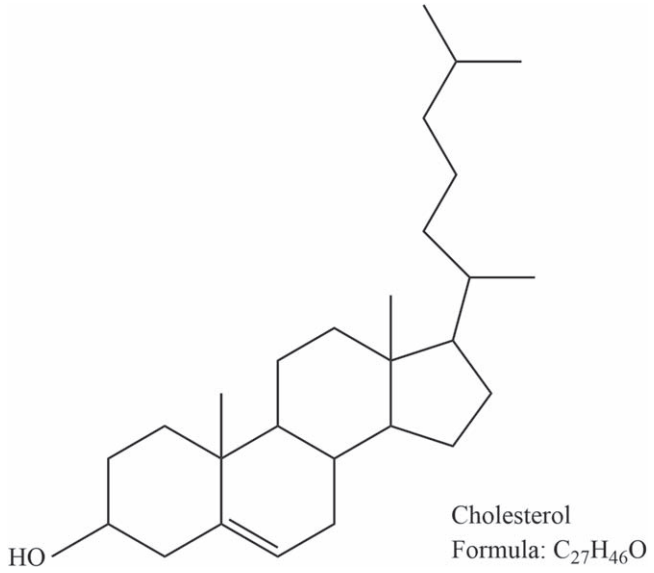

Figure 1. Structure of cholesterol.

analysis of cholesterol in clinical samples and food. These techniques are accurate and sensitive for precise quantitation of cholesterol. However, they depend on costly and large equipment and extensive sample pretreatment, which is also not suitable for the quick, convenient and economical detection in POCT.[46] On the contrast, since the invention of enzyme electrodes by Leland C. Clark, in 1962,[50] the enzymatic assays have been rapidly developed, evolving from costly, unstable laborious laboratory testing to the rapid-responsive, sensitive, stable and user-friendly analysis.[51] Furthermore, owning to inexpensive and miniaturized detection systems, colorimetric and electrochemical detection have become the optimal methods for POCT. Thus, these methods are also well explored in the research studies and are even commercialized in industry.

In this review, we summarize the enzymatic strip detection methods of serum total cholesterol for POCT devices in detail, including colorimetry and electrochemical detection. Moreover, new techniques and recent improvements are introduced. For the outlook, challenges and future trends in enzymatic detection methods for POCT are discussed.

Current Techniques

The enzymatic strip detection of serum total cholesterol for POCT devices.—With the quick development of sensing technologies for cholesterol detection, various detecting methods have been explored including surface plasmon resonance,[52] fluorescence,[53] colorimetry,[54] and electrochemistry detection,[55] etc. Among these methods, colorimetry and electrochemical detection methods have the merits of low cost, high effectiveness and well-acceptance, which are attracting and desirable for POCT applications. Thus, these two methods still prevail in numerous researches and commercialized products for POCT in the past decades.

Colorimetry strip.—As the science of color quantification, colorimetry is essential for color reproduction technology. In the early nineteenth century, the study was begun with the work of Young, Helmholtz and Maxwell, who recognized the principles of additive and subtractive colour mixing, and proposed the trichromatic nature of human colour vision.[56] In 1931, the science became formalized, and further developed with the recommendation of the Commission International de l'Eclairage (CIE), which the system about color specification based on the three tristimulus values X, Y and Z (XYZ). The current CIE reference represents more practical information on colorimetric measurement.[56] Colorimetry method is the very traditional and classical method for enzymatic assays. The aim of this method is to numerical describe the colors quickly and accurately. It is particularly attractive for point-of-use applications because the measured results can be simply read out in a single sheet of paper by unaided eyes, or through the absorbance of the color produced. Perception of the color is governed by three factors: the nature of the illumination, the optical properties of the object itself and the response of the human eye. Typically, colorimetry quantitates these aspects to unambiguously define color using three primary colors (red, green and blue, RGB).[56,57]

Cholesterol in the blood comes in two forms, free cholesterol (about 30%) and cholesteric esters (about 70%). The typical principle of enzymatic cholesterol colorimetric detection is shown as reaction Eqs. 1–3. At first, esterified cholesterol is hydrolyzed to free cholesterol by cholesterol ester hydrolase/cholesterol esterase (CEH). Subsequently the free cholesterol is oxidized to cholest-4-en-3-one and hydrogen peroxide (H_2O_2) by cholesterol oxidase (COD/ChOx). H_2O_2 is produced in this reaction as a side product and can be easily detected using high sensitive colorimetric probes. The producing H_2O_2 results in increasing intensity of color produced with increasing concentration of analyte via oxidation of the dyes such as 4-aminoantipyrine (4-AAP), o-dianisidine (ODA), 4-aminophenazone (APZ) etc, which are often utilized for cholesterol content estimation.[58]

$$Cholesterol\ ester + H_2O \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acid \quad [1]$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholest - 4-en - 3 - one + H_2O_2 \quad [2]$$

$$2H_2O_2 + 4 - Aminoantipyrine + Phenol \xrightarrow{Peroxidase} Quioneimine\ dye + 4H_2O \quad [3]$$

Similarly, in the reaction (3) where the color products is formed, phenol can be replaced by phenolic substances, such as 2,4-dichlorophenol, 3-hydroxy-2,4,6-triiodobenzoic acid (HTIB), etc and aniline substances, such as N-ethyl-N-(2-hydroxyl-3-sulfopropyl)-3-methyl aniline (TOOS), N-ethyl-N-(2-hydroxyl-3-sulfopropyl)-3,5-dimethoxy aniline (DAOS), etc.[59] Due to the conspicuous color, the chromogenic reagent 3,3,5,5-tetramethylbenzidine (TMB) is often used as a substrate for H_2O_2 detection in the presence of peroxidase to produce a blue color reaction.[60]

This traditional colorimetric method has already been recommended by Chinese Society of Laboratory Medicine (CSLM).[61] It shows high precision and accuracy. However, the specificity of this method may be impaired a little because cholesterol oxidase may also react with other sterols.[21,50] And some chemicals present in a sample (such as ascorbic acid and bilirubin) may consume H_2O_2. Thus, some bias during indirect cholesterol quantification may occur. Besides, it uses three-enzymes during the process, which have to be maintained bioactivities at the same time. Moreover, some inherent disadvantages such as low-stability, high-costs as well as difficult preparation largely hindered its wide application. As it is known peroxidase is needed in H_2O_2 detection.[61,62] Therefore, looking for efficient enzyme mimics is becoming an increasingly important goal in bioassays and medicine diagnostics.[60,63,64] Enzyme mimics possess many advantages over natural enzymes such as low cost, stability against denature or protease digestion.[62,65] Thus, it becomes an optimal option for enzymetic assays.

Since the emergence of artificial enzymes from 1965,[66] various materials, such as metal/metal oxide nanoparticles,[67,68] micro/nanomaterials,[66] and biomolecules.[69,70] and so on, have been extensively explored to mimic the structures and functions of natural enzymes through various approaches. In the recent decades, enzyme mimics have been applied in the detection of cholesterol.[60,62,71,72] Typically, Narsingh R. Nirala etc reported a

nanomaterial, graphene quantum dots (GQDs) which can mimic the enzyme of horseradish peroxidase (HRP), for unprecedented detection of free cholesterol.[62] Without the use of sophisticated instruments, the proposed method perceives the cholesterol using naked eye with blue compound formation. It could be made into strips. By contrasting with the color wheel, the concentration of cholesterol can be obtained. The result (Table I) showed that the proposed detection system based on GQDs allowed a wide range (0.02–0.6 mM) of cholesterol sensing with a detection limit as low as 0.006 mM along with high maximum initial velocity (V_{max}) and low Michaelis–Menten constant (K_m) value, suggesting that high catalytic activity, due to larger surface area of the GQDs. The research showed a great potential for enzyme mimics. But it still needs further study on the stability and detection of serum total cholesterol. What's more, the disadvantages, such as the complex preparation process, long response time and high cost, should be reminded. Yapeng Li etc prepared MXene-Ti_3C_2/CuS nanocomposites using a simple hydrothermal approach. The formation process needed to be heated at 160 ℃ for 24 h. The nanocomposites showed intrinsic peroxidase-like activity in comparison with individual MXene and CuS nanomaterials.[60] With satisfied selectivity, in the presence of COD and TMB, the MXene/CuS nanocomposites-based colorimetric sensor showed an outstanding performance for cholesterol determination with a broad linear range of 10–100 μM and a limit of detection (LOD) of 1.9 μM (Table I), which was more sensitive than many other reported methods. It showed a good linearity using serum ($R^2 = 0.9921$). However, the detective process of the colorimetric sensor in this study is closely associated with pH, temperature and H_2O_2 concentration, which required a high-standard production process for strips. Vinay Sharma and Shaikh M. Mobin demonstrated the use of a cytocompatible CuO:Graphene nanosphere (CuO:GNS) composite as a peroxidase mimic for detection of H_2O_2 and free cholesterol. The nanocomposite-based sensor presents excellent detection sensitivity for cholesterol and demonstrates a linear response ($R^2 = 0.99$) in the range of 0.1–0.8 mM with LOD as low as 78 μM. And the CuO:GNS was found to have better cytocompatibility than that of CuO only. More tests on real samples such as whole blood should be carried out for total cholesterol detection in further studies.[71] On the other respect, by adding biomolecule, Ruimin Li etc developed a novel colorimetric method for detection of cholesterol with hemin-G-quadruplex DNAzyme, which can transduce the oxidation of cholesterol into the color change of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$).[72] Oligonucleotide 5′-GTGGGTA GGGCGGGTTGG-3′(Oligo-1) can form G-quadruplex structure in the presence of K^+. It can act as a HRP mimicking DNAzyme when binding hemin, and can catalyze the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ by H_2O_2. Even though this assay showed good linearity ($R = 0.998$) using serum sample, the process was complicated. At present, most of those material-based studies are still staying in the lab and require further refinements for real-life applications. So further research on whole blood sample was needed. In general, nanomaterials combined with metal/metal oxide have shown better results in cholesterol detection, which could be seen as a direction for the future. Nevertheless, preparation process remains to be simplified.

Another important aspect in colorimetric method is the supporting material selection. The initial system worth mentioning is constructed on a single sheet of paper.[76] Cholesterol from the sample reacts with CEH and COD from the subsequent pad. Later H_2O_2 formed upon this reaction passes to the measurement pad. The measurement pad is uniformly modified with aforementioned dyes. The length of the pad that changes colors upon reaction is proportional to the cholesterol concentration in the sample.[43] As shown in Fig. 2, firstly the sample was dropped into the hole of the test paper. After reaction, we could see the color change by reversing the paper pad.

In addition, the materials of the paper pad itself can vary, which can have different influences for the speed and uniformity of color rendering.[43] To overcome these influences, Manasi Dhawane etc developed chitosan nanofiber-based cholesterol biosensor, involving colorimetric detection of the analyte.[51] The uniform and bead-free chitosan nanofibers fabricated by electrospinning, were utilized for immobilizing cholesterol oxidase and peroxidase enzymes. It revealed that the intensity of color change was a reflection on cholesterol concentration. In the research, pieces of the optimized nanofibrous mat (1 × 1 sq. cm) were made to detect concentration of cholesterol. The result showed good linearity ($R^2 = 0.99$). And the nanofiber used for the pad has a high surface to volume ratio, chemical stability and alterable porosity and malleability, which are desirable properties for biomedical sensing. This kind of material is urgently needed for mass production in companies at present. For further development, more research should be done in order to broaden the detection range and lower cost etc.

Lastly, colorimetric sensors have received significant attention especially in POCT due to the rapid determination of the analytes, low-cost and wide application. The colorimetric biosensors are composed of bio-elements and biomarkers, porous membranes for support and biomarker immobilization, and a signal generating system.[51] However, there are some problems in signal generating system. For example, the Accuchek Instant plus instruction card had several flaws. Most of the participants are not able to insert the test strip on their first attempt probably because the instruction card has no indicator for the correct side of the test strip to insert. After performing a finger stick the users are asked to apply a hanging drop of blood at the center of the test strip. The major problem within this test kit is the large amount of blood required for the test. Participants had to fill a well until the black circle was completely covered. Most of the participants were unable to squeeze out enough blood to complete the step. Besides, the participants must wait for 2–4 min or even longer before performing the next step. Some of the participants did not wait for enough time before proceeding to the next step, which could also lead to the detection failure.[77] In order to shorten the detection time, the research on cholesterol detection using microfluidic technology has been widely concerned in recent years. Mohammed A. Al-Rawhani etc realized the detection for total cholesterol quantification in pure blood serum by using a colorimetric complementary metal oxide semiconductor (CMOS)-based platform.[74] It could measure cholesterol concentration in human blood serum as low as 29 μM with LOD at 13 μM, which is approximately 400 times lower than average physiological level. However, the serum need be pre-treated with CEH for 10 min at 37 ° C to release free cholesterol from the esterified form. And the detection range is from 29 μM to 231 μM, which is relatively narrow. After that, in 2019 Mohammed A. Al-Rawhani etc presented a versatile single CMOS chip, which integrated interleaved sensing subsystems for quadruplemode colorimetric, chemiluminescent, surface plasmon resonance and hydrogen ion measurements.[78] And it could detect glucose, cholesterol, urea and urate. Usually, to directly detect cholesterol in whole blood, a filter such as a plasma separation membrane (PSM) should be assembled because the color of the blood cells interferes with the colorimetric assay. Sungsu Park group [79] and Fang Li group [75] developed PSM-integrated 3D-paper-based microfluidic analytical devices (μPADs) and double-layered 3D-μPADs separately. As demonstrated in the research, the 3D-μPADs could detected multiple substances simultaneously. As shown in Table I, the detection range of cholesterol is 0.01–0.4 mmol l^{-1}, and the detection limits was 0.07 μmol/L. The detection time is short (70 s). As a direction, more attention should be paid to the connecting issue between detection strips and signal generating system and the interference of the blood cells in the following study.

To eliminate the effects of ambient light, researches on signal generating system are proposed. For example, magnetic nanoparticles-based optical detection system depends on the aggregation of initially dispersed particles to yield a straightforward color change from yellow to brown,[8] which is easily to form uniform color products. Furthermore, a method of smartphone-based POCT

Table I. Sensing characteristics along with those reported in literature.

Enzyme	Chromogenic agent	Buffer	Detection range	Response time	Stability	References
COD/POD	4-AAP,3-methyl-2-benzothiazolinone hydrazine,N,N-dimethylaniline	Phosphate-buffered pH = 7.0		<15 min	6 months, 37 ℃	73
COD/ DNAzyme	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$)	10 mM Tris–Ac buffer (pH 8.0)	1.0–30 μM	—	—	72
COD/CuO:CNS	Phenol, 4-AAP	20 mM PBS	0.1–0.8 mM			71
COD/GQDs	3,3,5,5-tetramethylbenzidine (TMB)	0.15 M PBS, (pH = 7.0)	0.02–0.6 mM	5 min	—	62
CEH/COD/MXene-Ti_3C_2/CuS	3,3,5,5-tetramethylbenzidine (TMB)	0.5 mM Britton-Robinson (B-R) buffer solution (pH 7.4)	100–1000 μM	2 min	—	60
CEH/COD/POD	3,3′,5,5′-tetramethylbenzidine hydrochloride	PBS, 0.01 M, pH 7.4	50–300 mg dl^{-1}	5 min	—	51
CEH/COD/POD	o-Dianisidine	Triethanolamine, pH = 7.5	29–231 μM	—	—	74
CEH/COD/POD	luminol	Phosphate-buffered pH = 7.5	0.01–0.4 mmol l^{-1}	70 s	—	75

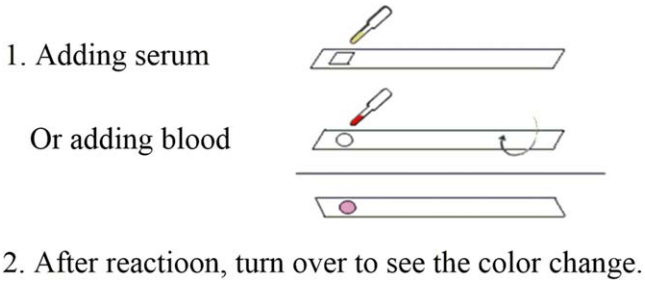

Figure 2. The use of traditional test strip.

urinalysis under various conditions of illumination was proposed,[9] which is convenient and adaptable to social trends. These methods could be considered in detection other samples, such as serum total cholesterol.

Electrochemical test strip.—Electrochemical method is the most frequently applied in the fabrication of enzyme-based cholesterol biosensors. A POCT device with an electrochemical sensor exhibits high precision, fast response, low cost, and portability.[3,80–84]

Based on the different enzymes used, it can be classified as two methods: cholesterol oxidase method [85,86] and cholesterol dehydrogenase method.[87,88] The basic principles of the reaction process are also introduced as follows (Figs. 3a and 3b).

As shown in Fig. 3b, to avoid the interference of reducing substances in blood (such as ascorbic acid etc) in cholesterol oxidase method, Yuzo Kayamori etc developed an endpoint assay for serum cholesterol, based on a CEH-cholesterol dehydrogenase (CDH)-ultraviolet method. However, there was a disadvantage at equilibrium, in which the CDH reaction gave incomplete conversion of cholesterol to cholest-4-ene-3-one (Fig. 3b). To overcome this disadvantage, hydrazine monohydrate, which is poisonous, should be added to the reaction mixture to remove cholest-4-ene-3-one, which allows the reaction to proceed to completion and gives stoichiometric production of β-NADH from the reaction of β-NAD^+ with cholesterol. So the CEH-COD-peroxidase chromogenic method is still widely used.

Apart from the enzyme-based classification, the electrochemical detection methods for cholesterol detection can be also divided based on different reaction principles: the consumption of oxygen and the rate of H_2O_2 formation by an enzyme reaction. Although the former is not disturbed by bilirubin and ascorbic acid, H_2O_2 is easier to transfer electrons. So the detection of cholesterol content is mostly reported by detecting the rate of H_2O_2 formation in recent years.[89]

Another classification method based on the recording techniques: the amperometric method and the voltammetric method. The amperometric total cholesterol measurement is performed based on the reactions (1, 2, 4) that occur on the surface of the working electrode.

$$H_2O_2 \xrightarrow{\text{Electrocatalytic oxidation}} O_2 + 2H^+ + 2e^- \qquad [4]$$

Reaction (4) produces an electric output current that can be recorded via the computer-controlled data acquisition software.[90]

Ksenia V. Derina etc used voltammetric sensor to detect total cholesterol. In their study it suggests that voltammetry, as an analytical technique, presents higher accuracy than the amperometric methods.[46] They fabricated a novel sensor by co-immobilizing COD and HRP on the porous graphite surface. It exhibits certain selectivity for the analyte in the presence of other compounds present in the sample including ascorbic acid, glucose, lactic acid, and uric acid. To evaluate the performance of voltammetric method, more studies should be carried out and further results should be obtained. At present the amperometric method is more widely used due to its convenience. The following are various studies based on amperometric method.

For the electrochemical sensors, the electrodes are usually fabricated using screen-printing technique. The typical electrodes are shown in Fig. 4a.[91] It is a two-electrode system, including a working electrode and a reference electrode. The electrodes are fabricated on the substrates, and then the reagents are immobilized in the reaction area. With the increasing demand for higher sensitivity and selectivity, three-electrode system and four-electrode system have been provided. The supporting or the counter electrode is added in the three-electrode system (Fig. 4b).[86] And hematocrit correction electrode is added in the four-electrode system in extra (Fig. 4c). The blood is inhaled into the test paper by siphon and passes through the conductance measurement area. The conductance test electrodes are composed of the trigger electrode and the second pair of electrodes measuring the hematocrit of blood flowing through the conductance measurement area. Then the blood enters the electrochemical measurement reaction chamber for total cholesterol determination. The measured value of total cholesterol is calibrated by using the hematocrit (Hct) value, based on the influence curve of Hct on blood glucose concentration.[92] Finally, the value is recorded and displayed on the tester.

For detecting systems, it should be considered in terms of sensitivity, LOD, fast response time, etc The initial electrochemical method for detection cholesterol requires high voltage to produce an electric current.[93] However, as the biological fluids such as serum contains high concentrations of other analytes, protein, etc, it is desirable to develop a sensing platform which can be operated at low voltages so as to prevent ChOx simultaneously reacting with other electroactive analytes in the samples, for example, uric acid, ascorbic acid, bilirubin, pyruvate, glutathione and produce false positive signals as well. That is why the second-generation enzymatic biosensor is widely used, which requires low voltages owing to the involvement of the electron mediator.[46]

To minimize the effect of interferences, techniques have been developed using the redox mediator ferricyanide to reduce the reaction potential of hydrogen peroxide. In order to avoid unnecessary oxidation of other electroactive analytes and obtain high sensitivity, the working potential of 300 mV is appropriately used.[91] In recent years, to further improve the sensitivity of those biosensors, some cholesterol electrochemical biosensor researches are focusing on the direct electron transfer between ChOx and electrode surfaces.[46,94–99] Sheela Berchmans' group and Shelley D. Minteer's group used $Ni(OH)_2$/NiOOH[100] and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, free radical)[101] as redox catalysts to mediate electron transfer respectively. They both showed excellent catalytic properties toward hydrogen peroxide. And the former one possessed long term stability and wide linear range, as shown in Table II. TEMPO, as an organic redox catalysts, could be used to monitor H_2O_2 in broad pH range (⩾4) at 530 mV, which was beneficial to specially detect cholesterol and could be used for various populations with different pH in blood. Due to the high potential required, Nidhi Chauhan etc use pencil graphite electrode (PGE) bounded cholesterol oxidase as working electrode, silver/silver chloride (Ag/AgCl) as reference electrode and Cu wire as auxiliary electrode. It has the advantage over previous amperometric methods that it requires low potential to generate electrons from H_2O_2, which does not induce ionization of serum substances.[47] COD is immobilized on PGE through chemisorption, a weak Van der Waals forces. Moreover, the current value is well correlated ($R = 0.99$) with the serum cholesterol concentration by standard enzymatic colorimetric method. The detection range was wide, which could be seen in Table II. Besides, no interference by metabolites was observed in the method. It may provide a path for the study of for the anti-interference effects using the serum or blood.

On the other hand, in theory, negative potential detection method is put forward for its little interference and the benefit for the human body. So it becomes another trend for the researchers. Pedro Salazar etc described a situ electrodeposition of ChOx-modified polydopamine (PDA) thin film on nanostructured-screen printed electrodes

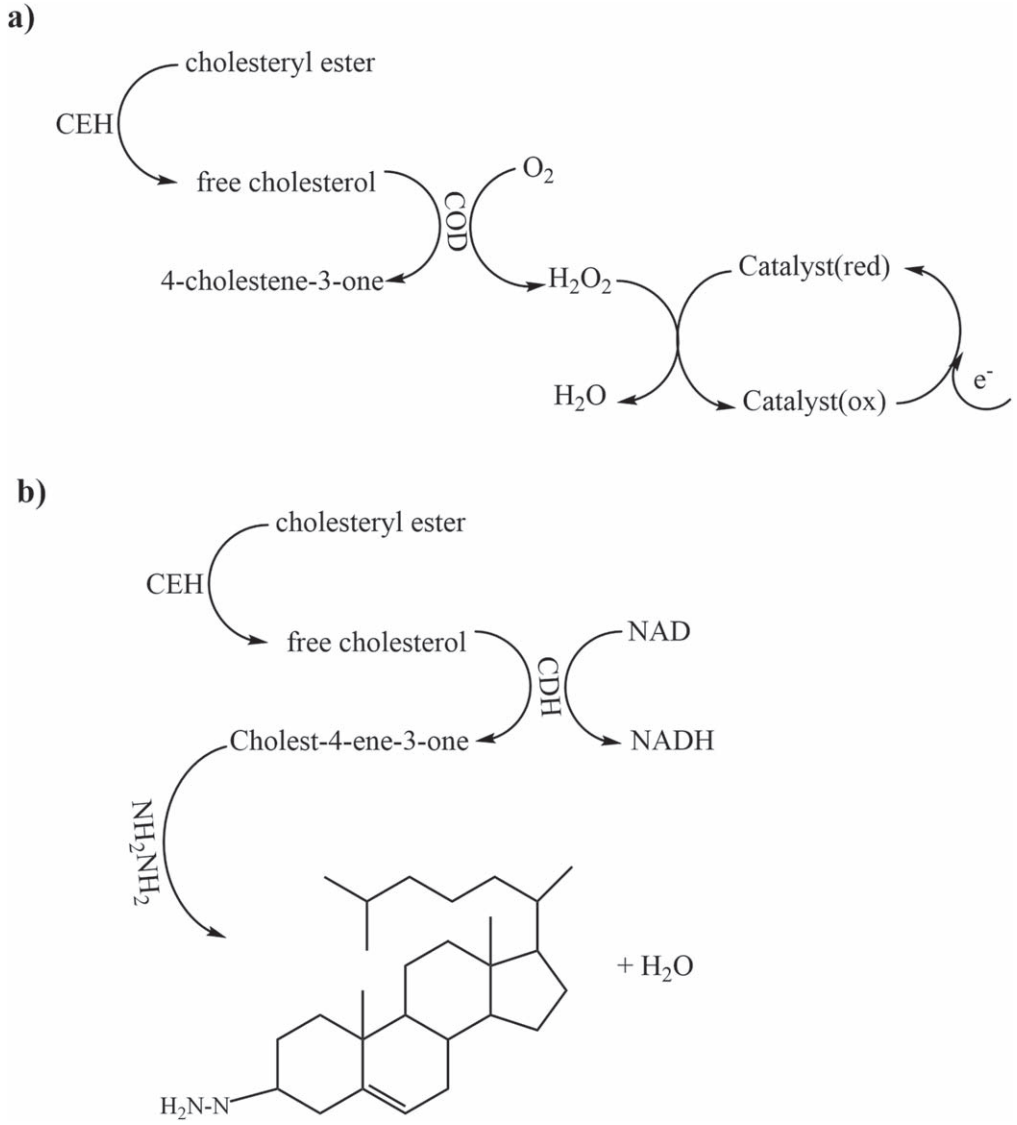

Figure 3. The reaction sequences of cholesterol oxidase method (a) and cholesterol dehydrogenase method (b).

(SPEs).[102] The applied potential was fixed to −0.1 V against the internal Ag pseudo-reference electrode. According to the reaction 1, 2 and 5, electrical current was produced.

$$[PW]Fe_4K_4(Fe(CN)_6)_3 + 2H_2O \rightarrow [PB]Fe_4K_4(Fe(CN)_6)_3 + 4OH^- + 4K^+ \quad [5]$$

It showed a rapid response after cholesterol addition, reaching the stationary current after ca. 7 s. The interfering substances, including ascorbic acid, glucose, acetaminophen, uric acid, fructose, sucrose and lactose, produced negligible effects on the actual cholesterol current, thus confirming the high selectivity for cholesterol detection. Prussian Blue (PB) and multi-wall carbon nanotubes (MWCNTs) were used to form a SPE/PB/MWCNTox/PDA@ChOx electrode as working electrode, and silver as pseudo-reference and counter electrode. Totally, compared with other electrodes,[103–106] it showed rapid response time, good anti-interference performance, high sensitivity. To be fair it has relatively narrow detection range (Table II). The preparation process should also be further simplified.

In order to optimize the detecting system, the immobilization for the enzyme is another important part that worth exploring. Since the activity of enzymes is closely related with pressure, temperature and pH, the immobilization of enzyme is crucial in order to maintain its activity.

Immobilization methods can be classified based on different standards. From the aspects of support, it can be divided into support-requiring and support-free. According to the nature of linkage between enzyme and the supporting material, it can be also classified into categories such as adsorption-based, covalent binding and cross-linking, entrapment and encapsulation.[43] The adsorption method (Fig. 5a) is simple, inexpensive and requires no chemical modification of enzymes. Unfortunately, this type of immobilization can result in subsequent leaching. At the same time, the distribution of the enzyme in paper matrix is difficult to control.[107] Besides, this method is also highly influenced by the reaction/deposition conditions, like pH that will impact the surface charge. Covalent binding (Fig. 5b) rigidifies structure of the enzyme.[108] If the enzyme is deactivated, the support, sometimes costly, is also rendered useless. In the case of entrapment (Fig. 5c) enzymes are typically enclosed in a polymer matrix (organic polymer, sol-gel, which is yellow in Fig. 5c). In this case the substrate is consecutively exposed to polymer solutions of the opposite charge. Immobilization of enzyme is achieved by electrostatic interactions or hydrogen bonds in some cases.[109] Encapsulation (Fig. 5d) results in similar characteristics to

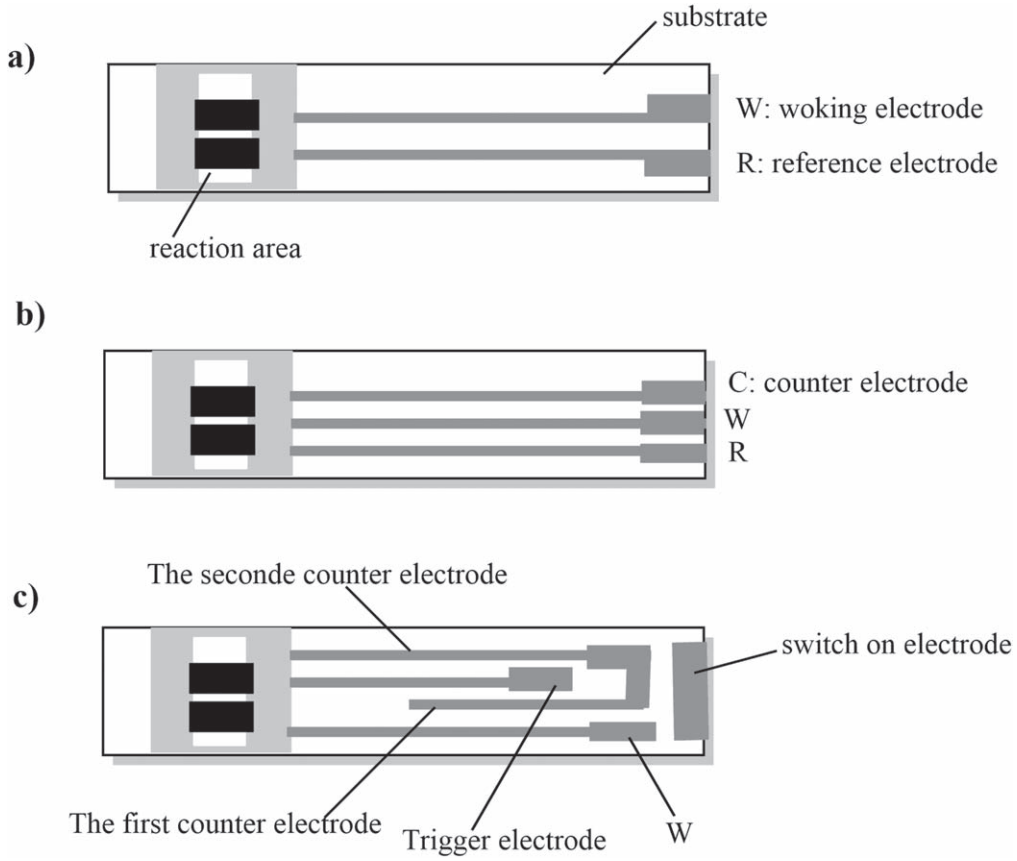

Figure 4. Schematic diagram of (a) two-electrode system, (b) three-electrode system, (c) multielectrode system.

entrapment. In this case, the immobilization could be carried independently in an optimized environment.[43] And many engineered enzymes with cellulose binding domains have already been commercially available, but the price was still high.[104,110]

Huseyin Bekir Yildiz etc designed two types of amperometric cholesterol biosensors by physically entrapping cholesterol oxidase in conducting polymers, thiophene capped poly(ethyleneoxide)/polypyrrole (PEO-co-PPy) and 3-methylthienyl methacrylate-cop-vinyl benzyloxy poly(ethyleneoxide)/polypyrrole (CP-co-PPy). The results showed that ChOx immobilized in PEO-co-PPy displayed higher affinity towards the substrate.[104] But it required high potential (0.7 V). M. Alagappan etc added conductive material ($K_4[Fe(CN)_6$) to lower the potential. They immobilized the ChOx on gold nanoparticles-functionalized-MWCNT-PPy nanocomposite modified electrode. PPy acts as a support matrix to hold ChOx and the presence of Au-f-MWCNT increases the electrical conductivity. However, the positive potential couldn't prevent the interference reaction.[106] Saniye Soylemez etc designed a simple and robust cholesterol biosensor by immobilizing ChOx onto a conducting polymer modified graphite electrode. Monomer, (Z)-4-(4-(9H-carbazol9-yl) benzylidene)-2-(4-nitrophenyl) oxazol-5(4H)-one (CBNP) was synthesized. After electropolymerization, it was used to immobilize the enzyme. As shown in Table II, it still had some disadvantages, such as narrow detection range, poor stability, complex process and so on.[105]

Based on the analysis of the above two methods, detection system has also been advanced to be disposable together with the test strip. However, this kind of one-component integration of electrochemical devices is still not available for a reasonable price. Instead, colorimetric system can realize equipment-free quantitative detection, which can be accomplished by appropriate design of the device. For example, cell phone cameras and downloadable software could also be used by simply installing appropriate softwares for color-intensity-based quantification. From this respect, colorimetric method is superior to electrochemical method. In summary, for low-cost applications in real-life situations, colorimetric or electrochemical system should be further improved to own the properties including self-contained and one-component design, using unprocessed sample, no specialized training required and easy to interpret.[43]

In conclusion, the key point of the cholesterol detection is to obtain the methods with low cost, simple preparation process and easy mass production under the premise of high sensitivity, good linearity, strong anti-interference ability, good stability, wide detection range and short response time.

Future Directions

In recent years, the concept of home-care has entered the public vision. Apart from glucose, pregnancy and international normalized ratio (INR) monitoring, the content of home care can be extended when new tests for chronic diseases become readily available. This important trend is termed as "personalized medicine".[3,80,111] In this case, chronic diseases (e.g., diabetes or atherosclerosis) may get early diagnosis compared with the traditional clinical approach, which is focused on the patient's clinical signs and symptoms. The therapeutic strategies would then be tailored to each patient individually, thus improving the therapeutic effects.

With increasing incidence of cardiovascular diseases and cardiac arrest due to the unhealthy diet, high work-load and pressure, cholesterol testing becomes essential for early diagnostics and daily monitoring. Meanwhile, the quick development of POCT has resulted in wide applications and a large investment. In further commercialization, those POCT-based cholesterol testing techniques must be scientifically grounded, evidence based and financially viable,[1] especially in developing countries. Financial viability is strongly associated with the healthcare policies of the respective national reimbursement system in most countries. For example, the

Table II. Sensing characteristics along with those reported in literature.

Ampere/voltage method	Enzyme(Biosensor)	Conducting polymer	Immobilize agent	Buffer	Working potentail	Blood volume	Detection range	Response time	Stability	References
Amperometric method	CEH/COD/POD	potassium ferrocyanide	CMC solution	PBS, pH = 7.0	0.3 V	2 μl	100–400 mg dL^{-1}	180 s	—	91
Amperometric method	COD/POD	—	—	PBS, pH = 6.8	0.1V		1.29–10.33 mM	30 s	25 days	47
Voltammetric method	COD/HRP	—	—	PBS, pH = 6.86	—	—	−300 M	—	15 days	46
Amperometric method	SPE/PB/MWCNTox/PDA@ChOx	[PW] $Fe_4K_4(Fe(CN)_6)_3$	PDA film electrodeposition	PBS, 0.1 M, pH 7.4	−0.1 V	—	5–400 μM	7 s	30 days	102
Amperometric method	Pt/PAn/ChOx	—	polyaniline film	PBS, pH = 7.02	0.6 V	—	10–100 μM		—	103
Amperometric method	CP-co-PPy/ChOx/Pt foil	—	PEO-co-PPy and CP-co-PPy	PBS, pH = 7.0	0.7 V	—	—		30 days	104
Amperometric method	poly(CBNP)/ChOx/graphite	—	CBNP	PBS, pH = 7.0	−0.7 V	—	2.5–27.5 μM		15 days	105
Amperometric method	AuNPs-f-MWCNT-PPy-ChOx/GCE	$K_4[Fe(CN)_6]$	polypyrrole (PPy)	PBS, pH = 7.0	0.3 V	—	2–8 mM	50 s	7 days	106
Amperometric method	Ru-Pi/PPy/CFP electrode	$K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$	—	PBS, pH = 7.0	−0.63 V	—	0.16–20.0 nM	—	30 days	97
	ChOx /SPE	luminol				20 μl	10–5000 μM	120–150 s		98
Amperometric method	GC/Cu-Pt-Bi /ChOx			PBS, 0.1M, pH = 7.2	0.30 V		0.05–8.8 mM	8 s	30 days	99
Amperometric method	GC/Chi-$Ni(OH)_2$/Chox/NF	—	chitosan	PBS, pH = 7.0	0.6 V	100 μl	0.45–10 mM		70 days	100
	ChOx/TEMPO/GCE			PBS, pH = 7.4	0.53 V		0.02–2.5 mM			101

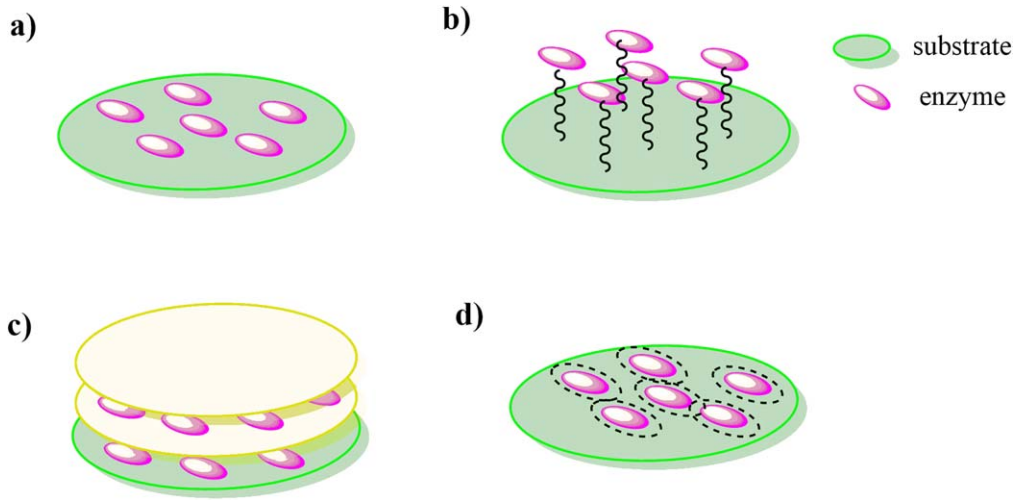

Figure 5. Immobilization methods using (a) adsorption, (b) covalent linkage, (c) entrapment, (d) encapsulation.

Third National Cholesterol Education Program (NCEP-III) was defined in term of specific lipid and lipoprotein target values. It proposed that "Lipoprotein profiles should be assessed at least annually and preferably at each clinic visit to promote compliance."[28,112] And since 2015, China has issued a series of relevant policies in the field of precision medicine, accelerating the follow-up of industry supervision and policy direction guidance, which will boost the development of diagnostic industry.

In the development of POCT based devices, many new techniques are involved to make it more convenient and user-friendly. As proposed by Steven R. Steinhubl etc, smartphone-based POCT devices can be used to monitor the various physical and molecular activities.[55,113,114] And Haipeng Yang' group described a stretchable hybrid film electrode composed of carbon nanotube and styrene-butadiene-styrene block copolymer.[115] The prepared stretchable biosensor to be proved was suitable to be equipped on new generation of wearable devices which can track the biochemistry signals such as sweat glucose, cholesterol and lactate, etc, which provided a new crossover idea between detection of cholesterol and wearable devices. Moreover, "Telemedicine" is also booming in this area. Instruments can send the data obtained directly to the relevant medical service point. When critical indicators exceed in values, the medical service can intervene for further medical examination. In this way it facilitates the early diagnostics and benefits the daily monitoring especially for chronic diseases. The effectiveness of home monitoring and thus patient safety are improved.[3,9] Based on the social needs, minimally-invasive micro-dialysis systems have become an optimal approach for a continuous mode of POCT measurements.

However, only a few new scientific payoffs of biosensor have been successfully launched in the market. One of the challenges lays in the optimization of critical parameters, such as enzyme stabilization, quality control and instrumentation design. The cholesterol biosensor must be easy to use, self-testing, fast response and portable, etc.[58] Only a few companies, such as Roche (US Pat. Nos. 6866675 and 4869249), Biosafe (US Pat. No. 6040135), Sinocare,[116] Aconbio,[117–119] etc, have the ability to obtain the techniques and commercialize their research. Increased understanding of the immobilized bioreagents, improved techniques for immobilization and technological advances in the microelectronics are likely to speed up the commercialization of the pressing-needed cholesterol biosensor.

In conclusion, nowadays the serum total cholesterol detection system is still in research more than in application. Because of the high cost, the application in industry is still limited. Therefore, on the one hand, more translation of scientific research into enterprises is urgently in need. On the other hand, researchers are required to further explore the low-cost and convenient techniques for mass production. Furthermore, although the LOD are usually low, the enzymatic strip detection of serum total cholesterol depends on costly enzymes for POCT devices. On the premise of high quality, how to lower the cost of the materials, such as thee carrier in colorimetric method, the immobilize agent and conducting polymer in the electrochemical method, is the main issues. What's more, industry standards for cholesterol testing should be established similarly as the glucose detection. Finally, nondestructive testing, real-time detection (wearable products), smartphone-based detection device and telediagnosis system are urgently required further improvement, which can provide portable and in-time approaches for health monitoring and early diagnosis.

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References

1. P. ST-Louis, *Clin. Biochem.*, **33**, 427 (2000).
2. B. Saboo et al., *Diabetes Metab. Syndr*, **13**, 5 (2019).
3. P. B. Luppa, C. Müller, A. Schlichtiger, and H. Schlebusch, *TrAC, Trends Anal. Chem.*, **30**, 887 (2011).
4. M. Boyd and T. Woolley, *Surgery (Oxford)*, **34**, 91 (2016).
5. R. L. Sautter, D. M. Earnest, and D. C. Halstead, *Clin. Microbiol. Newsl.*, **40**, 191 (2018).
6. J. Hutton et al., *Int. J. Drug Policy*, **72**, 84 (2019).
7. V. H. Buss, L. S. Deeks, A. Shield, S. Kosari, and M. Naunton, *Res. Social Adm. Pharm.*, **15**, 483 (2019).
8. Y. Xianyu, Q. Wang, and Y. Chen, *TrAC, Trends Anal. Chem.*, **106**, 213 (2018).
9. J. Liu, Z. Geng, Z. Fan, J. Liu, and H. Chen, *Biosens. Bioelectron.*, **132**, 17 (2019).
10. Y. Li, Z. Li, H. Liu, S. Chen, X. Guo, M. Lin, and F. Li, *J. Electrochem. Soc.*, **166**, B524 (2019).
11. A. Pietikainen, J. Oksi, and J. Hytonen, *Diagn. Microbiol. Infect. Dis.*, **91**, 226 (2018).
12. P. K. Drain, K. A. Heichman, and D. Wilson, *Lancet. Infect. Dis.*, **19**, 794 (2019).
13. H. Chen, K. Liu, Z. Li, and P. Wang, *Clin. Chim. Acta*, **493**, 138 (2019).
14. N. Mandal, M. Bhattacharjee, A. Chattopadhyay, and D. Bandyopadhyay, *Biosens. Bioelectron.*, **124–125**, 75 (2019).
15. H. Gao, L. Wen, J. Tian, Y. Wu, F. Liu, Y. Lin, W. Hua, and G. Wu, *Biosens. Bioelectron.*, **142**, 111504 (2019).
16. B. Kuswandi, A. A. Gani, and M. Ahmad, *Food Biosci.*, **19**, 1 (2017).
17. D. M. Jenkins, B. E. Lee, S. Jun, J. Reyes-De-Corcuera, and E. S. McLamore, *J. Electrochem. Soc.*, **166**, B3056 (2019).
18. H. I. Gomes and M. G. Sales, *Biosens. Bioelectron.*, **65**, 54 (2015).
19. N. S. K. Gunda, S. H. Gautam, and S. K. Mitra, *J. Electrochem. Soc.*, **166**, B3031 (2019).
20. J. Xu, *A saliva test paper of three programs for drug detection and preparation method*, CN 105527422 A (2016).
21. G. Chen and D. Li, *Multifunctional sampling detection stick for saliva, DNA sample collection and alcohol*, CN 108414512 A (2018).
22. J. Yang, K. Wang, H. Xu, W. Yan, Q. Jin, and D. Cui, *Talanta*, **202**, 96 (2019).
23. L. H. Li, E. P. Dutkiewicz, Y. C. Huang, H. B. Zhou, and C. C. Hsu, *J. Food Drug Anal.*, **27**, 375 (2019).

24. V. Narwal, R. Deswal, B. Batra, V. Kalra, R. Hooda, M. Sharma, and J. S. Rana, *Steroids*, **143**, 6 (2019).
25. L. Y. Jiang, F. F. Yao, Q. H. Chen, X. P. Xie, and G. Z. Cui, *Sensor World*, **16**, 6 (2010).
26. H. Z. Chen and X. J. Jin, *Eng. Sci.*, **4**, 1 (2002).
27. J. Stamler, M. L. Daviglus, D. B. Garside, A. R. Dyer, P. Greenland, and J. D. Neaton, *J. Am. Med. Assoc.*, **3**, 311 (2000).
28. R. C. Pasternak, *ACC Curr. J. Rev.*, **11**, 37 (2002).
29. D. M. Lloyd-Jones, L. T. Braun, C. E. Ndumele, S. C. SmithJr, L. S. Sperling, S. S. Virani, and R. S. Blumenthal, *J. Am. Coll. Cardiol.*, **73**, 3153 (2019).
30. Y. Zhu, J. M. Lu, Z. B. Yu, D. Li, M. Y. Wu, P. Shen, H. B. Lin, J. B. Wang, and K. Chen, *Nutr. Metab. Cardiovasc. Dis.*, **29**, 1205 (2019).
31. T. Bulum, M. Tomic, and L. Duvnjak, *Arch. Med. Res.*, **48**, 467 (2017).
32. E. Olie, M. C. Picot, S. Guillaume, M. Abbar, and P. Courtet, *J. Affect. Disord.*, **133**, 234 (2011).
33. S. L. Kraav et al., *Psychiatry Res.*, **272**, 326 (2019).
34. D. P. Forbes and G. E. Herman, *J. Lipid Res.*, **52**, 6 (2011).
35. M. Cassader, *Atherosclerosis*, **1**, 99 (1993).
36. W. Hong, V. Zimmer, Z. Basharat, M. Zippi, S. Stock, W. Geng, X. Bao, J. Dong, J. Pan, and M. Zhou, *Clin. Nutr.*, **39**, 250 (2019).
37. M. Martín, F. Pfrieger, and C. Dotti, *EMBO Rep.*, **15**, 1036 (2014).
38. X. Yu, T. J. Kelley, H. J. Chiel, and J. D. Burgess, *J. Electrochem. Soc.*, **163**, B453 (2016).
39. L. S. Eberlin, A. L. Dill, A. B. Costa, D. R. Ifa, L. Cheng, T. Masterson, M. Koch, T. L. Ratliff, and R. G. Cooks, *Anal. Chem.*, **82**, 3430 (2010).
40. A. Weiss, Y. Beloosesky, H. Schmilovitz-Weiss, E. Grossman, and M. Boaz, *Clin. Nutr.*, **32**, 533 (2013).
41. W. M. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 97 (1950), http://cel.webofknowledge.com/InboundService.do?customersID=atyponcel&smartRedirect=yes&mode=FullRecord&IsProductCode=Yes&product=CEL&Init=Yes&Func=Frame&action=retrieve&SrcApp=literatum&SrcAuth=atyponcel&SID=7A3YCfCNQLLndBuQPgD&UT=WOS%3AA1950UB75600013.
42. K. L. Ahuja and K. L. Bajaj, *J. Am. Oil Chem. Soc.*, **57**, 250 (1980).
43. E. Witkowska Nery, in *Analysis of Samples of Clinical and Alimentary Interest with Paper-based Devices* (Springer International Publishing, Switzerland) 25 (2016).
44. R. W. Burke, B. I. Diamondstone, R. A. Velapoldi, and O. Menis, *Clin. Chem.*, **20**, 794 (1974), https://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=21&SID=7APzIhSu6nIiBcmVt9D&page=1&doc=1.
45. M. Nakamura et al., *Clin. Chim. Acta*, **445**, 127 (2015).
46. K. V. Derina, E. I. Korotkova, E. V. Dorozhko, O. A. Voronova, and D. A. Vishenkova, *Procedia Chem.*, **10**, 513 (2014).
47. N. Chauhan, J. Narang, and C. S. Pundir, *Am. J. Anal. Chem.*, **01**, 41 (2010).
48. A. Ahmadalinezhad, A. K. M. Kafi, and A. Chen, *Electrochem. Commun.*, **11**, 2048 (2009).
49. T. Ferri, A. Poscia, and R. Santucci, *Bioelectrochem. Bioenerg.*, **44**, 177 (1998).
50. M. M. Niraj, H. M. Gupta, S. Varshney, S. Pandey, and S. Singh, *Int. J. Ther. Appl.*, **6**, 28 (2012), https://www.scopus.com/record/display.uri?eid=2-s2.0-84927510232&origin=resultslist#.
51. M. Dhawane, A. Deshpande, R. Jain, and P. Dandekar, *Sensors Actuators B: Chem.*, **281**, 72 (2019).
52. S. K. Arya, P. R. Solanki, S. P. Singh, K. Kaneto, M. K. Pandey, M. Datta, and B. D. Malhotra, *Biosens. Bioelectron.*, **22**, 2516 (2007).
53. H. C. Chang and J. A. A. Ho, *Anal. Chem.*, **87**, 10362 (2015).
54. N. R. Nirala, S. Pandey, A. Bansal, V. K. Singh, B. Mukherjee, P. S. Saxena, and A. Srivastava, *Biosens. Bioelectron.*, **74**, 207 (2015).
55. M. Zarei, *Biosens. Bioelectron.*, **98**, 494 (2017).
56. A. Gilchrist and J. Nobbs, in *Encyclopedia of Spectroscopy and Spectrometry*, ed. J. C. Lindon, G. E. Tranter, and D. W. Koppenaal (Academic Press, Oxford) 3rd ed. (2017).
57. A. D. Broadbent, in *Encyclopedia of Spectroscopy and Spectrometry*, ed. J. C. Lindon, G. E. Tranter, and D. W. Koppenaal (Academic Press, Oxford) 3rd ed. (2017).
58. S. K. Arya, M. Datta, and B. D. Malhotra, *Biosens. Bioelectron.*, **23**, 1083 (2008).
59. Z. Huangwu and J. Jincheng, *Stabilization of cholesterol oxidase*, CN 104245931 A (2013).
60. Y. Li, Z. Kang, L. Kong, H. Shi, Y. Zhang, M. Cui, and D. P. Yang, *Mat. Sci. Eng. C*, **104**, 110000 (2019).
61. C. S. O. L. Medicine, *J. Chin. Med. Exam.*, **18**, 185 (1995), http://g.wanfangdata.com.cn/details/detail.do?_type=perio&id=QK199500896608.
62. N. R. Nirala, S. Abraham, V. Kumar, A. Bansal, A. Srivastava, and P. S. Saxena, *Sensors Actuators B: Chem.*, **218**, 42 (2015).
63. M. I. Kim, J. Shim, T. Li, J. Lee, and H. G. Park, *Chemistry (Easton)*, **17**, 10700 (2011).
64. H. J. Cheon, M. D. Adhikari, M. Chung, T. D. Tran, J. Kim, and M. I. Kim, *Adv Healthc Mater*, **8**, e1801507 (2019).
65. Q. Wang, Z. Yang, X. Zhang, X. Xiao, C. K. Chang, and B. Xu, *Angew. Chem., Int. Ed. Engl.*, **46**, 4285 (2007).
66. H. Wei and E. Wang, *Chem. Soc. Rev.*, **42**, 6060 (2013).
67. Y. Liu, G. Zhu, J. Yang, A. Yuan, and X. Shen, *PLoS One*, **9**, e109158 (2014).
68. C. L. Hsu, C. W. Lien, C. W. Wang, S. G. Harroun, C. C. Huang, and H. T. Chang, *Biosens. Bioelectron.*, **75**, 181 (2016).
69. S. J. Pollack, J. W. Jacobs, and P. G. Schultz, *Science*, **234**, 1570 (1986).
70. Y. Lu, N. Yeung, N. Sieracki, and N. M. Marshall, *Nature*, **460**, 855 (2009).
71. V. Sharma and S. M. Mobin, *Sensors Actuators B: Chem.*, **240**, 338 (2017).
72. R. Li, C. Xiong, Z. Xiao, and L. Ling, *Anal. Chim. Acta*, **724**, 80 (2012).
73. M. P. Allen, A. DeLizza, U. Ramel, H. Jeong, and P. Singh, *Clin. Chem.*, **36**, 1591 (1990), https://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=16&SID=7APzIhSu6nIiBcmVt9D&page=1&doc=1.
74. M. A. Al-Rawhani et al., *IEEE Sens. J.*, **17**, 240 (2017).
75. F. Li, J. Liu, L. Guo, J. Wang, K. Zhang, J. He, and H. Cui, *Biosens. Bioelectron.*, **141**, 111472 (2019).
76. X. Li and X. Liu, *Microfluid. Nanofluid.*, **16**, 819 (2014).
77. D. Surabattula, C. M. Harvey, F. Aghazadeh, J. Rood, and A. Darisipudi, *Int. J. Ind. Ergon.*, **39**, 167 (2009).
78. M. A. Al-Rawhani, C. Hu, and C. Giagkoulovits et al., *IEEE*, **0018–9294**, C (2019).
79. C. Park, H. R. Kim, S. K. Kim, I. K. Jeong, J. C. Pyun, and S. Park, *ACS Appl. Mater. Interfaces*, **11**, 36428 (2019).
80. E. Stürenburg and R. Junker, *Dtsch. Arztebl.*, **106**, 48 (2009).
81. S. A. Atty, A. H. Ibrahim, and E. M. Hussien, *J. Electrochem. Soc.*, **166**, B1483 (2019).
82. A. M. Abdel-Aziz, H. H. Hassan, A. A. Hassan, and I. H. A. Badr, *J. Electrochem. Soc.*, **166**, B1441 (2019).
83. G. Li, P. Zhong, Y. Ye, X. Wan, Z. Cai, S. Yang, Y. Xia, Q. Li, J. Liu, and Q. He, *J. Electrochem. Soc.*, **166**, B1552 (2019).
84. M. M. Hasan, M. A. Ehsan, T. Islam, N. H. Alharthi, H. F. Alharbi, M. R. Karim, M. A. Aziz, and A. J. S. Ahammad, *J. Electrochem. Soc.*, **166**, B1528 (2019).
85. M. Situmorang, P. W. Alexander, and D. B. Hibbert, *Talanta*, **49**, 639 (1999).
86. W. C. Shih, M. C. Yang, and M. S. Lin, *Biosens. Bioelectron.*, **24**, 1679 (2009).
87. Y. Kayamori, H. Hatsuyama, T. Tsujioka, M. Nasu, and Y. Katayama, *Clin. Chem.*, **45**, 2158 (1999), https://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=19&SID=7APzIhSu6nIiBcmVt9D&page=1&doc=1.
88. T. Quah, S. Abdellaoui, R. D. Milton, D. P. Hickey, and S. D. Minteer, *J. Electrochem. Soc.*, **164**, H3024 (2017).
89. R. Gulaboski, V. Mirceski, R. Kappl, M. Hoth, and M. Bozem, *J. Electrochem. Soc.*, **166**, G82 (2019).
90. Y. Yoneyama, Y. Yonemori, M. Murata, H. Ohnuki, K. Hibi, T. Hayashi, H. Ren, and H. Endo, *Talanta*, **80**, 909 (2009).
91. G. Li, J. M. Liao, G. Q. Hu, N. Z. Ma, and P. J. Wu, *Biosens. Bioelectron.*, **20**, 2140 (2005).
92. J. Guo and L. Shi, *The invention relates to a high precision total cholesterol electrochemical paper with hematocrit compensation*, CN 109270142 A (2018).
93. A. A. Ivanov, N. V. Yudina, E. I. Korotkova, and O. I. Lomovsky, *Solid Fuel Chem.*, **42**, 68 (2008).
94. A. Merkoçi, M. Pumera, X. Llopis, B. Pérez, M. del Valle, and S. Alegret, *TrAC, Trends Anal. Chem.*, **24**, 826 (2005).
95. J. J. Gooding, *Electrochim. Acta*, **50**, 3049 (2005).
96. S. Moussa and J. Mauzeroll, *J. Electrochem. Soc.*, **166**, G25 (2019).
97. K. B. Akshaya, A. Varghese, M. Nidhin, and L. George, *J. Electrochem. Soc.*, **166**, B1016 (2019).
98. D. Wang, C. Liu, Y. Liang, Y. Su, Q. Shang, and C. Zhang, *J. Electrochem. Soc.*, **165**, B361 (2018).
99. V. C. Soorya and S. Berchmans, *J. Electrochem. Soc.*, **164**, B435 (2017).
100. K. Selvarani and S. Berchmans, *J. Electrochem. Soc.*, **164**, H561 (2017).
101. S. Abdellaoui, K. L. Knoche, K. Lim, D. P. Hickey, and S. D. Minteer, *J. Electrochem. Soc.*, **163**, H3001 (2016).
102. P. Salazar, M. Martín, and J. L. González-Mora, *J. Electroanal. Chem.*, **837**, 191 (2019).
103. H. Wang and S. Mu, *Sensors and Actuators B*, **56**, 22 (1999).
104. H. B. Yildiz, D. O. Demirkol, S. Sayin, M. Yilmaz, O. Koysuren, and M. Kamaci, *Journal of Macromolecular Science, Part A*, **50**, 1075 (2013).
105. S. Soylemez, Y. A. Udum, M. Kesik, C. Gündoğdu Hızlıateş, Y. Ergun, and L. Toppare, *Sensors Actuators B: Chem.*, **212**, 425 (2015).
106. M. Alagappan, S. Immanuel, R. Sivasubramanian, and A. Kandaswamy, *Arabian J. Chem.*, **13**, 2001 (2018).
107. U. Hanefeld, L. Gardossi, and E. Magner, *Chem. Soc. Rev.*, **38**, 453 (2009).
108. L. Cao, *Curr. Opin. Chem. Biol.*, **9**, 217 (2005).
109. D. Brady and J. Jordaan, *Biotechnol. Lett*, **31**, 1639 (2009).
110. R. Pelton, *TrAC, Trends Anal. Chem.*, **28**, 925 (2009).
111. R.-I. Stefan-van Staden, R.-M. Ilie-Mihai, L. A. Gugoasa, and C. Stanciu-Gavan, *J. Electrochem. Soc.*, **166**, B183 (2019).
112. M. L. Dansinger, P. T. Williams, H. R. Superko, and E. J. Schaefer, *Prev. Med.*, **121**, 150 (2019).
113. J. Li, Y. Sun, C. Chen, T. Sheng, P. Liu, and G. Zhang, *Anal. Chim. Acta*, **1052**, 105 (2019).
114. S. R. Steinhubl, E. D. Muse, and E. J. Topol, *Sci. Transl. Med.*, **7**, 283rv3 (2015).
115. J. Zhuang, X. Jiang, J. Wang, C. Yang, and H. Yang, *J. Electrochem. Soc.*, **164**, H1028 (2017).
116. Z. Huang and X. Cai, *An electrochemical test strip and a method for simultaneously detecting a variety of objects*, CN 106353387 A (2016).
117. L. Tang and T. Shang, *Biological sample analysis detector*, CN 104897912 A (2012).
118. T. Liu, H. Ge, J. Wang, and Y. Ye, *An automatic coding device and a biosensor provided with the device and a manufacturing method.*, CN 102967636 A (2012).
119. T. Shang and C. Wang, *Test strip*, CN 102128918 A (2010).