Assessment of the lipoprotein profile with point-of-care tests: an overview

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***Abstract*—The lipoprotein profile is an insightful test providing information on total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. Existing POC lipid analyzers measure blood fats using disposable biosensor strips and an optical reader. In presence of the target measurand, the strip produces a color proportional to the analyte concentration, whose intensity is measured by reflectance photometry. This manuscript introduces the importance of cholesterol testing and presents an overview on the working principle of the the biosensor strips and on the accuracy criteria recommended for their commercialization.**

INTRODUCTION

Cardiovascular diseases (CVD) represent the first cause of death in the world, with more people dying annually from CVDs than from any other cause [1]. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths [1]; of these deaths, about 7.4 million were due to coronary heart disease (CHD). The most important behavioral risk factors of CHD are represented by unhealthy diet, physical inactivity, tobacco use and alcohol abuse. Behavioral risks manifest in individuals as raised blood pressure, glucose and blood lipids, overweight or obesity. These "intermediate risks factors" can be measured in primary care facilities and indicate an increased probability of developing a heart attack, stroke, heart failure and other complications [1].

In 1985, the National Heart, Lung, and Blood Institute of the United States of America launched the National Cholesterol Education Program (NCEP) to reduce the prevalence of increased blood cholesterol, thereby contributing to the reduction of CHD morbidity and mortality [2]; in 1988, the NCEP's Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, either said the Adult Treatment Panel (ATP) issued a report describing a national strategy to identify people at risk for CHD [3]. This national strategy is based on conclusive scientific evidence that controlling blood cholesterol and triglycerides reduces the risk for CHD [4], [5]. The association between high blood cholesterol and the risk for CHD has become one of the most widely known medical relationships among health care professionals and the general public [6] , increasing interest in cholesterol testing and focusing unprecedented attention on the need to provide reliable measurements of blood lipids.

It is recommended that everyone aged 20 or older should have their blood fats measured at least once every 5 years [7]. The lipoprotein profile (lipid panel) provides a good level of detail, delivering information on the status of: total cholesterol, LDL (bad) cholesterol – the main source arterial obstruction,HDL (good) cholesterol – which prevents cholesterol buildup in the arteries, and Triglycerides, another form of blood fats.

The results of lipoprotein profile are grouped in ranges corresponding to optimal, borderline, or high concentrations, giving a patient a first hint about his own health status (table 1). These guidelines however are not meant to replace the physician's clinical judgment, which determines the appropriate treatment for each patient considering all the clinical and diagnostic information available. This manuscript presents an overview of the working principle of the colorimetric point of care lipid tests, And introduce the accuracy criteria recommended for their commercialization.

OVERVIEW OF POINT-OF-CARE LIPID ANALYZERS

While the measurement of the lipid panel molecules (hereby called with the general terms *analyte* or *measurand*) is a blood exam routinely performed in a clinical lab, in the last years the market has seen the introduction of portable point-of-care (POC) testing devices for the assessment of the cholesterol levels at the doctor's office, in a pharmacy or at home using a drop of capillary blood. Such devices can play a crucial role in the prevention of CHD, since enable fast and inexpensive tests which can be used by professionals as screening base for complementary diagnostics tests, to decide a first therapeutic action or to follow up patients at risk. Existing point-of-care lipid analyzers measure blood fats using disposable biosensor strips and an optical reader. Briefly, in presence of the target measurand, the strip produces a color proportional to the analyte concentration, whose intensity is measured by reflectance photometry. The reflected light is

Table I: Medical decision points for blood lipids.[3], [8]

Total Cholesterol	
Concentration (mM)	*Status*
>5.18	Desirable
5.18-6.20	Borderline
>6.21	High
HDL Cholesterol	
<1.04	Low (bad)
1.05-1.55	Borderline
>1.55	Desirable
Triglycerides	
<2.20	Optimal
2.20-4.5	Borderline
>4.5	High
LDL Cholesterol	
<2.59	Optimal
2.59-3-35	Near optimal
3.36-4.12	Borderline
4.13-4.91	High
>4.92	Very High

then related to the measurand concentration through a linear regression equation. The sequence is illustrated in figure 1.

BIOSENSOR STRIPS WORKING PRINCIPLE

All the strips are composed by different layers containing the reagents necessary to the detection of the target compounds, as illustrated in figure 2.

The top layer of the strip collects uniformly spreads the blood on the test region; a second layer filters the cells from plasma, which migrates by capillarity to a fractionation layer which deliver the blood to the various biosensor systems. The reaction layer contains the enzymes and the reagent necessary to the analysis and performs a series of enzymatic reactions whose common goal is the production of hydrogen peroxide. The hydrogen peroxide acts a limiting agent in a final enzymatic reaction that converts a colorless compound into a colored dye. The amount of dye produced in each sensor pad is then used to quantify the presence of a total cholesterol, HDL cholesterol of Triglycerides with a reflectometric reading on the back of the strip, through the support layer. The general strip reaction can therefore be divided in three steps:

1) *Separation of blood cells from plasma through a filtration membrane*
2) *analyte + enzyme system* $\rightarrow H_2O_2$ *production*
3) *(limited)* H_2O_2 *+ excess dye + excess peroxidase* $\rightarrow$ *color generation proportional to the analyte concentration*

The detailed reactions occurring in each sensor pad are presented more in detail in table 2.

LDL cholesterol (LDL) is not directly measured with an enzymatic biosensor but calculated from the values of TC HDL and TG using the Friedwald's formula [10]:

$$LDL(\frac{mg}{ml}) = TC - HDL - \frac{TG}{5}$$

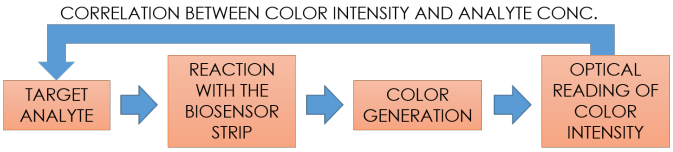

Figure 1: General sequence for the optical measurement of blood lipids using a biosensor strip and a reflectometer.

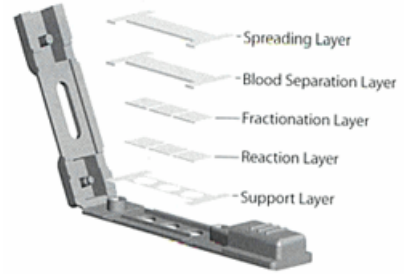

Figure 2: Layers of a colorimetric test strip for lipid panel measurements.

REFLECTANCE PHOTOMETER WORKING PRINCIPLE

The reflectance is the fraction of incident light reflected at a surface. When inserted in the reader device, the application area of the test strip is lit by an LED (light-emitting diode) from below, and the light reflected by the sensor strip is collected by a detector nearby (figure 3). The wavelength emitted by the led corresponds to the wavelength at which the light is adsorbed by the dye, hence higher dye concentrations will reflect less light. The test strips usually take from 60 to 180 seconds to form a stable color. The reflectance is measured at regular intervals until a plateau is reached. The last 5 measures are then averaged to calculate the final reflectance value. To normalize the results, the strips are measured before the contact with blood and after the stable dye formation, and the two values are subtracted. The reflectance value measured by the strips is correlated to the analyte concentration via a linear regression equation preloaded in the detector.

SYSTEM CALIBRATION, TRUENESS, PRECISION AND ACCURACY: GENERAL CONCEPTS

In order to correlate the reflectance reading to the analyte concentration, the strips are measured in our labs using solutions with predefined analyte concentrations in human serum. A regression equation is derived by the results, and unknown measurand values are then calculated by interpolation. The regression equations for total cholesterol, HDL cholesterol and triglycerides are obtained by the measurement of 10 different concentrations plus one blank, in five replicates. Lipid levels are chosen in order to uniformly cover the measurement range of the test strips; additional concentrations have been added across the medical decision points, in order to increase the calibration weight in such critical levels. The calibration curve for each analyte is shown in figure 3. The colored area represent the normal (green), borderline high (yellow) and high (red) concentrations ranges, as recommended by the ATP III [3]. Concentrations outside the graph represent values where the test system has lower accuracy. Beyond these points, the readings will be classified as "low" or "high"

Because each analysis is done on a single use strip, and strip behavior may slightly vary according to fabrication conditions or human manipulations, the accuracy of the detector is validated with a trueness and precision study. Bias studies assess the difference between samples value measured with the test strip and with reference methods granting extremely high measurement accuracy; precision studies evaluate how close the results obtained by similar tests are, evaluating the

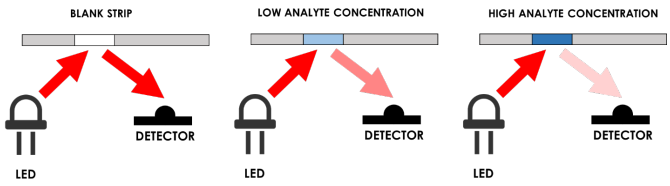

Figure 3: Working principle of reflectometric analysis: higher dye concentrations adsorb more light, decreasing the reflectance of the sensor strip.

Table II: enzymatic reactions of the test strips.

Total Cholesterol (TC)
$Cholesterol\,Ester + H_2O \xrightarrow{cholesterol\,esterase} Cholesterol + fatty\,acid$
$Cholesterol + H_2O + O_2 \xrightarrow{cholesterol\,oxidase} Cholesterol - 4 - en - 3 - one + + H_2O_2$
$2H_2O_2 + 4AAP + aniline \xrightarrow{peroxidase} Quinoneimine\,dye + 4H_2O$
HDL Cholesterol (HDL)
$Total\,cholesterol \xrightarrow{LDL\,and\,VLDL\,precipitation} Cholesterol + fatty\,acid$
$Cholesterol\,Ester + H_2O \xrightarrow{cholesterol\,esterase} Cholesterol + fatty\,acid$
$Cholesterol + H_2O + O_2 \xrightarrow{cholesterol\,oxidase} Cholesterol - 4 - en - 3 - one + + H_2O_2$
$2H_2O_2 + 4AAP + aniline \xrightarrow{peroxidase} Quinoneimine\,dye + 4H_2O$
Triglycerides (TG)
$Triglyceride + 3H_2O \xrightarrow{lipoprotein\,lipase} glycerol + 3\,fatty\,acid$
$glycerol + ATP \xrightarrow{glycerol\,kinase + Mg^{2+}} glycerol - 3PO_4 + ADP$
$glycerol - 3PO_4 + O_2 \xrightarrow{glycerol\,phosphate\,oxidase} Dihydroxyacetone - PO_4 + H_2O_2$
$2H_2O_2 + 4AAP + aniline \xrightarrow{peroxidase} Quinoneimine\,dye + 4H_2O$

Table III: CRMLN recommended certification criteria for cholesterol sensors *[9]*. No guidelines for triglycerides have been published so far.

Parameter	total cholesterol	HDL cholesterol	LDL cholesterol
r^2	>0.975 (linear regression)	>0.975 (linear regression)	>0.975 (linear regression)
bias at medical decision points	$\leq 3\%$	$\leq 5\%$	$\leq 4\%$
average % bias	$\leq 3\%$	$\leq 5\%$	$\leq 4\%$
average absolute bias	$\leq 3\%$	$\leq 5\%$	$\leq 4\%$
t-test of bias	not significant at $\alpha = 5\%$	not significant at $\alpha = 5\%$	not significant at $\alpha = 5\%$
total allowable error	$\leq 8.9\%$	$\leq 13\%$	$\leq 12\%$

closeness of results between different strips, days and users. Trueness and precision can be compared to hits in a target: high trueness correspond to hits very close to the target center, while high precision corresponds to hits very close to each other, regardless the distance from the center. The combination of trueness and precision yields the measurement accuracy. Accuracy values must fall within specific parameters before the system is released on the market. Table 3 shows the requirements recommended by the Cholesterol Reference Method Laboratory Network CRMLN.

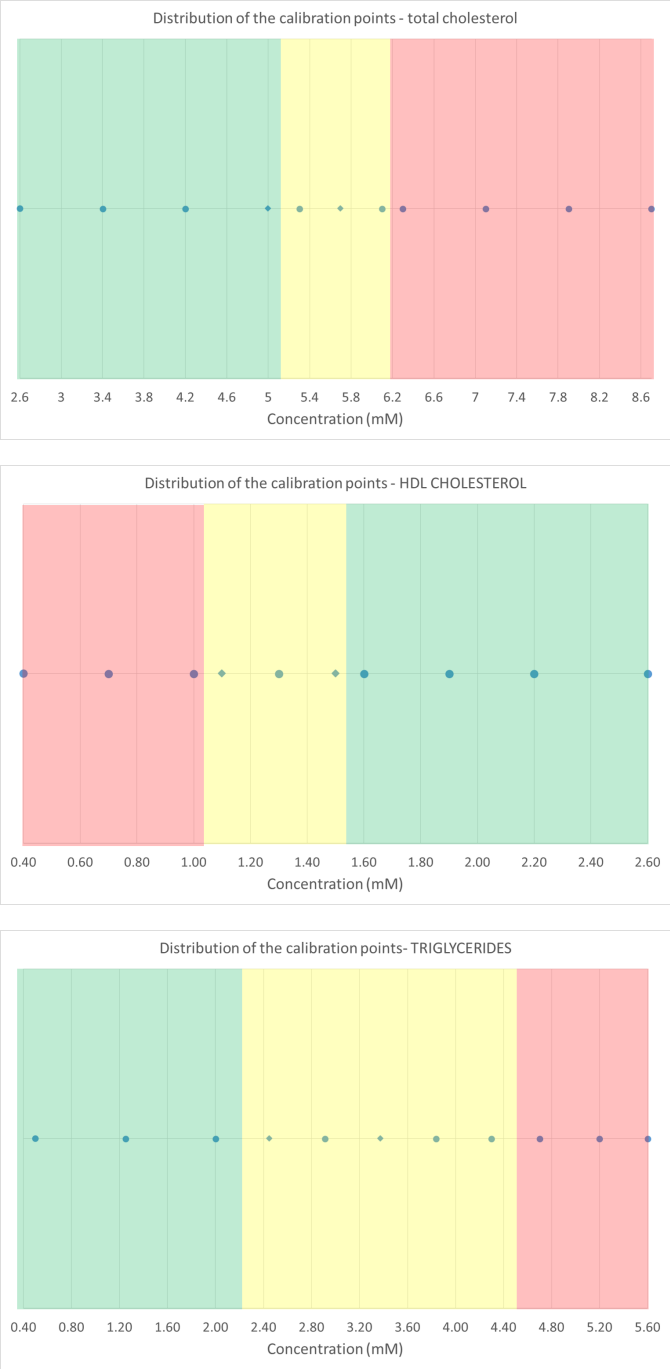

Figure 4: distribution of the calibration points.

CONCLUSIONS

Everyone aged 20 or older should have their blood fats measured at least once every 5 years. The lipoprotein profile is an insightful test providing information on total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. Existing POC lipid analyzers measure blood fats using disposable biosensor strips and an optical reader. In presence of the target measurand, the strip produces a color proportional to the analyte concentration, whose intensity is measured by reflectance photometry. Because each analysis is done on a single use strip, and strip behavior may slightly vary according to fabrication conditions or human manipulations, the accuracy of the detector is validated with trueness and precision studies. Accuracy values must fall within specific parameters before the systems are released on the market: the CRMLN network provides a series of guidelines that if satisfied provide quality results.

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