

Evaluation of an automated system for amylase detection in forensic samples

G. Da Vela^a, M.G. Bertino^a, F. Fusini^a, U. Ricci^{b,*}

^a *Laboratorio di Analisi Chimico Cliniche e Microbiologia,
Azienda Ospedaliera-Universitaria "A.Meyer", Florence, Italy*

^b *U.O. Genetica Medica, Azienda Ospedaliera-Universitaria "A.Meyer", Florence, Italy*

Abstract. Detection of the amylase enzyme in forensic samples is a very important step for the identification of the origin of a biological sample. The methods used in the forensic context employ chromatic reactions with visualisation of a colour and/or with spectrophotometric detection at an established wavelength. Here, we used an automated system which is normally employed in clinical chemistry laboratories to measure the amylase activity in forensic samples. This method is perfectly integrated with DNA typing procedure. A calibration curve for sensitivity study using a commercial amylase preparation was performed to verify the linearity range with semi-automatic and automatic methods. Visual evaluation of the results was also annotated. The automated method was employed for various samples containing human saliva (cigarette butts, chewing gum, stamps, etc.). We used this detection system also on biological samples containing human saliva that was contaminated with different materials, which are commonly recovered in forensic casework (ground, plaster, lipstick, glue). The sensitivity of the system is superior to the other methods and offers an objective evaluation of the amylase in forensic samples. © 2005 Elsevier B.V. All rights reserved.

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1. Introduction

Amylase identification in forensic samples is a very important step before performing DNA typing. High amylase levels in a fluid or stain suggest the presence of saliva and then of nucleated cells in a material, which could indicate success to detect the DNA of the donor/s. Moreover, the evidential value of the presence or absence of amylase activity in casework can help the investigator to rebuild the dynamic of a crime. Manual or semi-

* Corresponding author. Tel.: +39 0555662942; fax: +39 0555662931.

E-mail address: u.ricci@meyer.it (U. Ricci).

automated systems are reported to be normally used to identify this enzyme [1–3]. However, these methods are time-consuming and can be influenced by a large variety of factors affecting the substrate (colouring, dyes, dirtiness, etc.). Here, we evaluate the efficiency of an automatic chemistry system (Dimension[®] Dade Behring) on simulated forensic samples in comparison with a semi-automatic system. Our results show that the automated method results to be more sensible compared to the visual and semi-automated and offers an objective evaluation of the presence of amylase, also in old forensic samples.

2. Materials and methods

A Dimension[®] AR^X clinical chemistry system (Dade Behring) is a floor-model, microprocessor-controlled, integrated instrument/chemistry system that measures a variety of analytes, including enzyme activities, in body fluids. This system was employed in combination with Flex[®] reagent cartridge (AMY method) for automatic determination of amylase activity. α -amylase catalyses the hydrolysis of a defined synthetic substrate, 2-chloro-4-nitrophenyl- α -D-maltotrioside (CNPG3) in 2-chloro-4-nitrophenol (CNP), 2-chloro-4-nitrophenyl- α -D-maltoside (CNPG2), maltotriose (G3) and glucosio. After an incubation of 70 s at 37 °C, the absorption due to the formation of 2-chloro-4-nitrophenol (CNP) is measured using a bichromatic (405, 577 nm) rate technique.



For semi-automatic analyses a spectrophotometric system Lambda 11 (Perkin Elmer) was employed, in combination with a kinetics kit (BNP-Amylase test, Sclavo Diagnostics). A calibration experiment was performed with serial dilution of α -amylase from human pancreas (Sigma, cat. A9972) from 600 U/L to 1 U/L with both methods. Visual observation of the colour was also registered. Simulated forensic samples were prepared with 2 μ l of saliva mixed with ground, plaster, lipstick and glue. Three-year-old samples from yellow and white envelopes, chewing gum and cigarette butts were used. At each sample 500 μ l of isotonic solution (0.9% NaCl) was added, mixed and incubated at room temperature for 1–2 h. After centrifugation (5000 rpm/1 min) 100–200 μ l of the top

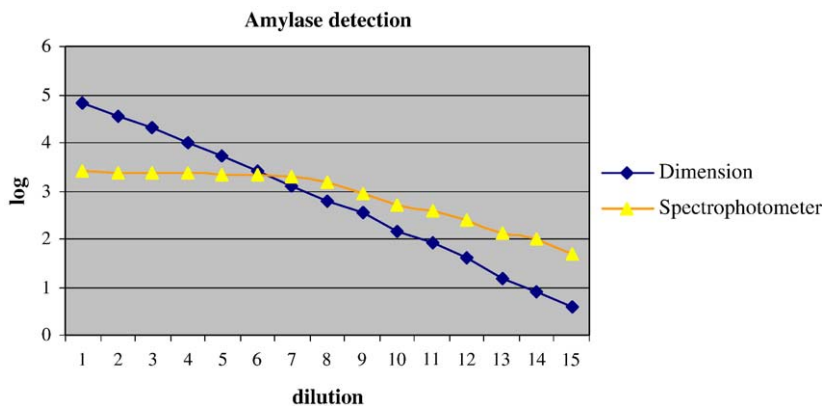


Fig. 1. Calibration curve for the clinical chemistry system and the semi-automated system.

solution were used for the tests. With both systems amylase activity was determined at 37 °C like suggested in the respective user manual [4,5].

3. Results

Fig. 1 shows the calibration standard curve for amylase with an automatic and semi-automatic method. The sensibility of the automatic system results to be better respect to that of the semi-automatic method. Moreover, since this clinical chemistry system uses a bichromatic rate technique, the amylase activity is not influenced by the substrate characteristic. In fact, all negative samples cut from the envelopes without saliva resulted negative with this test. All samples containing saliva were positive for the presence of amylase activity, with a range from 47 to 221 U/L for the old envelopes, 400–500 U/L for chewing gum and 20–30 U/L for the cigarette butts. Artificially contaminated saliva samples were positive for amylase to 55 U/L (ground) to 500 U/L (plaster). Using the semi-automated method, negative calibration of the system was necessary to detect the presence of amylase activity. Moreover, visual detection of yellow colour was negative under a dilution under 10 U/L of amylase standard. It is important to know that using these procedures evaluation of the results have to be performed within few minutes. Tubes left at room temperature for 30 or more minutes after adding the tests showed a yellow coloration also in negative samples.

4. Conclusion

Our experiments show that this clinical chemistry system is very useful to analyse forensic samples for amylase identification. Also coloured samples like cigarette butts can be efficiently analysed with this system, without the necessity of preparation of a negative control. This automatic system can give an objective valuation of the presence of amylase in a sample and can be efficiently integrated with a normal procedure used in DNA analysis. In fact, a wash step and a centrifugation was sufficient to isolate the cells at the bottom of the tube and the top phase can be directly used for determination of the amylase activity without disturbing the pellet. Also if this system is an *in vitro* diagnostic test intended for the quantitative determination of amylase activity in serum, plasma and urine, we found that it could be efficiently used for amylase identification in forensic samples.

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