TECHNICAL NOTE

The Recovery of Human Saliva Using the Salivette System

By P.-J. Lamey and Anita Nolan

School of Clinical Dentistry, Royal Victoria Hospital, Belfast, United Kingdom

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Summary: Human saliva recovery using the Salivette system was investigated. Two Salivette systems were employed. The recommended centrifugation procedures for saliva recovery only recovered between 46% and 52% of the total volume. Modification of centrifugation speeds and duration allowed an 80% recovery. Differences were observed between the two Salivette systems tested with the newer System allowing a greater recovery. The findings have implications for saliva collection using this technique.

Introduction

Numerous diverse ways of collecting saliva have been devised (1). Accurate methods are available for the collection of parotid saliva, submandibular/sublingual saliva and minor gland secretions. For collection of whole saliva several techniques have been used including expectoration of saliva and cotton wool weighing methods. In an attempt to provide a standard method for saliva collection, a system (Salivette — Sarstedt Leicester) was introduced in 1987 (2). The original System utilised a standard cotton roll (Sarstedt Leicester; 51-1534) and latterly a polyester coated insert (Sarstedt Leicester 51-1534-002) has also been introduced. We describe technical aspects of the recovery of human saliva from these Salivette systems.

Materials and Methods

Salivette containers were obtained from Sarstedt Ltd. Leicester. Two forms of the system were employed, either the plain cotton roll form (51-1534) or the new polyester insert form (51-1534-002). Mixed saliva samples (30 ml) were collected from three adult healthy volunteers (two male and one female) and the following experiments performed in triplicate. Saliva density was calculated by weighing the cotton roll Salivettes and adding known volumes of mixed saliva dispensed with a microsyringe. The density of saliva was on average 0.978 kg/l (range 0.976 — 0.979). The entire Salivette systems were also weighted prior to each of the experiments and had an average weight of 5.32 g.

Fifteen experiments were then performed adding 0.2 ml increments of mixed saliva to both the plain cotton roll as the polyester insert form up to a maximum of 3.0 ml. Having allowed the mixed saliva to be absorbed, the cotton rolls were returned to their insert and the stopper replaced. The Salivette tubes were then centrifuged at...
the recommended 600 g for 2 minutes (2) and at 2000 g for 2 minutes. On completion of this the fluid at the conical base of the tube was measured by removal with a microsyringe and the cotton inserts removed and reweighed. This procedure was repeated in triplicate for all volumes of mixed saliva dispensed.

The consistency in the density of the human saliva samples and of the weights of the Salivette system enabled subsequent experiments to be interpreted meaningfully.

Results

The percentage recovery of mixed saliva after centrifugation at 600 g for 2 minutes in relation to the volume of mixed saliva added to the plain cotton roll is shown in figure 1. This method only allowed recovery of between 46% and 52% (SD ± 7.2) of saliva and only then when volumes of added saliva were between 1.5 ml and 2.2 ml. The recoverable saliva using 2000 g for 2 minutes allowed saliva recovery from the plain cotton roll of 86% (SD ± 5.8) (fig. 2). It can be seen that added volumes of saliva in excess of 2.0 ml–2.2 ml is greater than the absorptive capacity of the roll using either recovery technique. The intersection of the percentage volume collected and percentage volume still retained by the cotton roll after centrifugation is around 1.6 ml at 600 g and 0.6 ml at 2000 g.

In contrast the results for the polyester insert system after centrifugation at 2000 g for 2 minutes are shown in figure 3. In this experiment the percentage volume recovered is regularly in excess of 80% (SD ± 6.3) of the total even when small mixed saliva samples were used.

Conclusion

This study has shown that if the Salivette system is to be used in clinical practice to assess whole salivary output, then it is essential that the polyester insert system is used in preference to the plain cotton roll. The recoverable saliva volumes with the polyester insert system are achieved at a lower loading volume of saliva and is maintained. In contrast the plain cotton system only achieves a recovery of around 80% and only then when the loading saliva volume is around 2.0 ml. The previously published suggestion method for the duration and speed of centrifugation with the plain cotton roll grossly underestimates the actual salivary volume retained within the roll. Additional studies are required to investigate the effect of either system on the binding of salivary constituents.

References


Professor P.-J. Lamey
School of Clinical Dentistry
The Queen's University of Belfast
Grosvenor Road
Belfast BT12 6BP
Northern Ireland (U. K.)
TECHNICAL NOTE

Comparison of a Time-Resolved Immunofluorimetric Assay with Two Immunoenzymatic Methods for α-Foetoprotein in Human Serum

By Maria Angela Bacigalupo, A. Ius, G. Meroni, L. Farina and C. Iacobello

1 Istituto Chimica Ormoni C. N. R., Milano, Italy
2 Bouty Italiana Laboratori, Sesto San Giovanni (Milano), Italy
3 Terzo Laboratorio Spedali Civili, Brescia, Italy

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Summary: We compared two commercial methods with a time-resolved immunofluorimetric assay for human α-foetoprotein in human serum using the europium complex of 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid as label. The correlation coefficients were $r_1 = 0.94$ and $r_2 = 0.96$.

Introduction

Human α-foetoprotein is a glycoprotein normally produced by the foetus, which diffuses through the amniotic membrane into the maternal circulation. In adults, levels of human α-fetoprotein may be high because of tumours of the liver, testis or ovary. During pregnancy, high levels of the analyte may indicate neural tube defects in the foetus (1), whereas low levels may be considered to be indicative of Down's syndrome (2). Radioimmunological methods are usually used to assay human α-foetoprotein, but there is increasing interest in developing immunological assays with non-radioisotopic labels. These include enzymatic, chemiluminescent (3-6) or fluorescent markers (7-9).

We have standardized a time-resolved immunofluorimetric assay (TR-IFMA) for human α-foetoprotein in human serum, using the europium complex of 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid as marker (10). The fluorescence of the europium complex was read in solution after dissociation from the solid phase (11). For clinical use the assay was precise and had a low detection limit, compared with other fluorescence immunoassays (12).

Materials and Methods

Materials

Polyclonal antibody anti-human α-foetoprotein, biotinylated monoclonal antibody anti-human α-foetoprotein and British standard human α-foetoprotein 72/227 were supplied by Bouty Laboratories (Milan, Italy). The microwell plates were obtained from Microstrip (Helsinki, Finland); streptavidin, bovine serum albumin and ovalbumin were obtained from Sigma (St. Louis, MO, USA). The other chemicals were purchased from Aldrich Chimica (Milan, Italy).

Apparatus

The Arcus model 1230 fluorometer with time-resolution (Wallac, Turku, Finland) was used for europium measurement.

Synthesis

The 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid was synthesized as described in I.e. (10). Streptavidin was labelled with 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid as described in I.e. (9).

Solid phase antibody preparation

The polyclonal antibody, anti-human α-foetoprotein, was adsorbed onto the wells of polystyrene microtitre strips. The wells were coated overnight at 27 °C with 250 μl of carbonate buffer (0.1 mol/l, pH 9.0) containing 30 mg/l of anti-human α-foetoprotein. After washing with carbonate buffer, the wells were saturated with the same buffer containing 10 g/l bovine serum albumin and 10 g/l ovalbumin.

Immunoassays

Commercial methods

1) Serum samples were assayed with the commercial enzyme immunoassay, AIA-PACK AFP, from Tosoh (Osaka, Japan), using the automatic analyser AIA-1200 from the same supplier. Inter-assay precision, given as the variation coefficient at three different concentrations (21.4, 103, 186 kIU/l), was respectively 4%, 3.6%, 3.7%.
2) Serum samples were also assayed with the BEIA AFP kit from Bonty Laboratories.

Interassay precision, given as the variation coefficient at three different concentrations (9.0, 40, 130 kIU/l), was respectively 9.4%, 7.5%, 7.3%.

**TR-IFMA**

A standard curve was prepared in duplicate, using a serial dilutions of the human α-foetoprotein standard in the BEIA AFP kit; 10 μl of standard containing 0—500 IU, or 10 μl of serum were pipetted into coated wells, followed by 90 μl of monoclonal biotinylated antibody diluted in Tris buffer (0.05 mol/l, pH 7.5, containing 9 g/l NaCl, 0.5 g/l Na₂, and 2 g/l bovine serum albumin).

After 1 hour at 37 °C, the wells were washed, and 100 μl of a dilution of streptavidin labelled with 4,7-bis(chlorosulphophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (30 nmol/1) were added to each well; the wells were kept at 37 °C for 30 min, then washed six times with 0.15 mol/l NaCl.

**Measurement of fluorescence**

After washing, 250 μl of dissociation solution (4 mol/l urea, 10 g/l sodium dodecylsulphate and 1 μmol/l europium trichloride) were added to each well. For both methods, fluorescence was measured after 20 min, using the excitation wavelength of 345 nm. The delay time was 400 μs after excitation, the emitted light being read at 615 nm.

The results are given in International Units (IU).

**Results and Discussion**

The standard curve and precision profile for time-resolved immunofluorimetric assay are shown in figure 1. The sensitivity of the method at twice the standard deviation was 0.5 kIU/l. The correlations between the three methods are reported in table 1.

Our method is not affected by contamination with europium and uses a stable and cheap dissociation solution. In addition, the labelled streptavidin is stable at 4 °C for at least one year, and lyophilized it is stable for at least two years.

Our results show that the TR-IFMA method has good sensitivity and is suitable for determination of human α-foetoprotein in serum at the very low screening concentrations for foetal Down's syndrome.

**References**


M. A. Bacigalupo
Istituto Chimica Ormoni C.N.R.
via Mario Bianco 9
I-20131 Milano
Italy