Table 1. Optimization of ASO hybridization for nonisotopic detection of point mutations in 21-OH deficiency.

Mutation[a]	Sequence (5′–3′)[b]	T, °C[c]	Fragment[d]
Pro 30 (N)	TCC ACC TCC CGC CTC TTG	51	*a* (ex 1)
Leu 30 (M)	TCC ACC TCC TGC CTC TTG		
i 2 A (N)[e]	AGC CCC CAA CTC CTC CT		
i 2 C (N)	AGC CCC CAC CTC CTC CT	49	*a, b* (in 2)
i 2 G (M)	AGC CCC CAG CTC CTC CT		
Ile 172 (N)	CAG CAT CAT CTG TTA CC	46	*b, c* (ex 4)
Asn 172 (M)	CAG CAT CAA CTG TTA CC		
ex 6 (N)	CAC ATC GTC GAG ATG CAG	45	*c* (ex 6)
ex 6 (M)	CAC AAC GAC GAG AAG CAG		
Val 281 (N)	AAG GGC ACG TGC ACA TG	49	*c* (ex 7)
Leu 281 (M)	AAG GGC ACT TGC ACA TG		
306 (N)	AGC AAA AAA ACC ACG GCC	49	*c* (ex 7)
306 insT (M)	AGC AAA AAA AAC CAC GGC		
Gln 318 (N)	AGC GAC TGC AGG AGG AG	51	*c* (ex 8)
Stop 318 (M)	AGC GAC TGT AGG AGG AG		
Arg 356 (N)	TGC GCC TGC GGC CCG TT	57	*c* (ex 8)
Trp 356 (M)	TGC GCC TGT GGC CCG TT		
Pro 453 (N)	CCC GGA GGG CAG CAG CGT	59	*c* (ex 10)
Ser 453 (M)	CCC GGA GGA CAG CAG CGT		

[a] Mutations in the coding region are given as amino acid in the normal (N) or mutated (M) sequence and its position in the protein, except for the splicing mutation at position 655 in intron 2, called "i2", followed by the base that is changed; the triple mutation in exon 6, called "ex 6"; and the frameshift mutation in exon 7 at nucleotide position 306, which is an insertion of a T (insT).

[b] Sequences (5′ to 3′) of the oligonucleotide pairs specific for the normal and mutated sequences. The bases that differ are underlined.

[c] Stringent washing temperature. Prehybridization, hybridization, and the nonstringent washing were performed at 42° C, for 2 h, 16 h, and 90 min, respectively. Two aliquots (15 and 5 mL) of 5 × SSC (20 × SSC: 3 mol/L NaCl, 0.3 mol/L sodium citrate) containing 10 g/L blocking reagent (Boehringer Mannheim), 1 g/L lauryl sarcosine, and 0.2 g/L sodium dodecyl sulfate were used in the pre- and hybridization steps, respectively. Stringent and nonstringent washes were made with 30 mL of 5 × SSC.

[d] PCR-amplified fragment/s in which the mutation is analyzed. In parentheses is given the number of the exon (ex) or intron (in) in which the mutation is located.

[e] Position 655 in intron 2 (see Fig. 1) has three alleles, two found in the normal population (A or C), and another (G) that is the splicing mutation in the mutated alleles. See text for identification of *a, b,* and *c.*

negative controls of each mutation (different PCR amplification, membrane, solutions, days, technicians) gave identical results.

Besides point mutations, the PCR-ASO protocol also allowed the characterization of some large conversions and deletions. Those conversion and deletions with one of the *CYP21B*-specific regions in their sequence are PCR-amplified and react positively to several mutated oligonucleotides *[6–9].*

The nonisotopic protocol has been as successful as the isotopic one for detection of point mutations, and the concordance of results has been total. Although slightly longer than the radioactive protocol, because additional incubations are required to develop the signal, the DIG protocol with the chemiluminescent substrate is safe, rapid, and sensitive and can be performed in all laboratories without special installations. Use of the chemiluminescent substrate permits reprobing of the membranes, which is important when several mutations or polymorphisms must be tested on the same amplified DNA fragment. In addition, the DIG 3′end-labeling has been performed on unmodified, previously synthesized oligonucleotides. Stock oligonucleotides synthesized for other purposes are suitable, and, more importantly, the labeled oligonucleotide is stable for months and ready for use when required.

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Immunoreactivity of Prostate-Specific Antigen in Plasma and Saliva of Healthy Women, *Ferdinando Mannello,[1]* Giuseppe Bianchi,[2] and Giancarlo Gazzanelli[1]* ([1] Ist. di Istol. ed Anal. di Lab., Facoltà di Sci. MFN dell'Università, Via E. Zeppi, 61029 Urbino, Italy; [2] Lab. Anal. dell'Ospedale Civile, Urbino, Italy; *author for correspondence: +39-722-320168)

Quantification of prostate-specific antigen (PSA) is widely used for early detection and monitoring of prostate cancer *[1],* even though numerous recent publications have unambiguously

Table 1. Plasma and saliva PSA values in women.

	Mean ± SE (and range), μg/L	
	Plasma	Saliva
Healthy controls	0.047 ± 0.008 (0.02–0.16)	0.048 ± 0.007 (0.02–0.15)
Contraceptive takers	0.045 ± 0.006 (0.02–0.12)	0.099 ± 0.016[a] (0.045–0.34)

[a] Significantly different by paired *t*-test from saliva concentrations of controls ($P = 0.0038$) and from their own paired plasma concentrations ($P = 0.0101$). n = 20 in each group.

shown that PSA is a widespread biochemical marker produced by many tumors, by normal tissues, and during pregnancy *[2–5]* and have pointed out the lack of specificity for prostate cells *[6]*. In light of evidence indicating the immunoreactivity of PSA in salivary gland neoplastic tissues *[7]*, we undertook to study the PSA distribution in plasma and expression in saliva of a control group of healthy female volunteers. The PSA determinations were carried out with a commercially available kit from CIS Bio International (Gif-sur-Yvette, France), based on a solid-phase two-site IRMA. The CIS PSA-RIACT assay utilizes two monoclonal antibodies, prepared against sterically remote sites of human PSA; the first is coated onto a test tube, the other is radiolabeled with ^{125}I, and both sandwich the PSA molecules of the samples. The radioactivity bound to the test tube is linear in the range 0–120 μg/L, with a lower detection limit of 0.02 μg/L *[8]*.

Blood and saliva were sampled from 20 apparently healthy women, ages 19–54 years (mean 34.3 years). Each participant was asked to rinse her mouth with water 5 min before collecting each sample. The saliva samples were centrifuged at 3500*g* for 15 min at 4 °C and the supernates were immediately frozen at −20 °C and thawed only once before assay. Blood samples were also drawn from these women at the time of the saliva collection and, after clotting, were centrifuged at 1500*g* for 15 min at 4 °C and then stored at −20 °C until assayed. Another 20 healthy women, ages 22–43 (mean 33.5 years), had been taking for at least the preceding 9 months a widely prescribed oral contraceptive containing 0.5 mg of gestoden (a progestin) and 0.035 mg of ethinylestradiol per tablet. The present work was carried out in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

All samples examined contained detectable amounts of PSA, with a median of 0.039 μg/L. No statistically significant difference in plasma concentration was found between the control women and the group that had been taking the hormonal contraceptive. We also found no statistically significant correlation between PSA content and the women's age, even after logarithmic transformation of data. Of the 40 women, 34 (85%) showed a plasma PSA concentration ≤0.06 μg/L—results in close agreement with those reported previously *[5]*. The PSA concentrations in saliva correlated significantly with the plasma concentrations in the healthy subjects ($r = 0.684$, $Z = 3.447$, $P = 0.006$), but in women taking progestin-containing oral contraceptives the saliva PSA content was significantly greater than the concentrations in plasma (see Table 1).

To our knowledge (after a careful review of the literature) this is the first report concerning the concentrations of PSA in saliva. A previous immunohistochemical study revealed a positive stain for this antigen in the brush border of ductal epithelial cells of normal salivary gland, as well as in several types of salivary gland tumors (i.e., adenoma/carcinoma of human salivary gland) *[7]*.

The detectable amounts of PSA in saliva from healthy women give further evidence of the distinctiveness of PSA as a widespread biochemical marker. The increase of oral-contraceptive-induced PSA expression is probably related to modulation of this serine protease by several hormones *[9, 10]*. The presence of progesterone receptors in human salivary gland *[11]* may represent one possible mechanism of increased stimulation of PSA expression in saliva of women receiving oral hormonal contraceptive.

The biological effects and the mechanism causing the increase, however, remain difficult to explain. The possible biological role of this serine protease in nonprostatic tissue as a potential sensitive molecular marker of hormone responsiveness of the glandular cells, as yet only hypothesized *[10]*, must be further investigated.

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Four Automated Methods for Plasma Lactate Assessed by Comparison with Capillary Gas Chromatography, *Klazina E. Brooks[1] and Norman B. Smith[1,2,*]* [[1] Dept. of Clin. Biochem., Univ. Hosp. Campus, London Health Sci. Centre, P.O. Box 5339, London, Ontario, Canada N6A 5A5 (address for correspondence); [2] Dept. of Biochem., Univ. of Western Ontario, London, Ontario, Canada; *author for correspondence: fax 519-663-3858; e-mail nsmith@julian.uwo.ca]

During an instrument consolidation process in our laboratory, we needed to identify our best analyzer for doing plasma lactate assays. However, a comparison of our *aca* V (Dupont Medical Products, Mississauga, Ont., Canada), Synchron CX5 (Beckman Instruments, Mississauga, Ont., Canada), TDx (Abbott Diag-