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Examination of the Correlation of Groupings in Blood and Semen

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ABSTRACT: The grouping of blood/saliva samples from a male so as to predict his semen groups is only justified if there is a strict correlation between the groupings in these body fluids. This correlation has been examined in the ABO, phosphoglucomutase (PGM_1) and glyoxalase I (GLO) grouping systems in blood and semen samples collected from more than 250 individuals. Though no results proved inconsistent with this correlation, a number of semen gave inconclusive grouping results. Reasons for this are discussed as well as the relevance of the results to semen stain analysis. Semen amylase activities are also reported.

KEYWORDS: pathology and biology, genetic typing, semen, saliva, blood, correlation, group, phosphoglucomutase, glyoxalase, ABO, amylase

When comparing the grouping results obtained from a crime-related seminal deposit with the groupings of suspects, reference semen samples from each suspect are normally unobtainable. Therefore reference blood and saliva samples from each suspect are grouped and the results used to predict their semen groups. This procedure rests on the assumption that as a principle of genetics, an individual will have the identical genetic polymorphisms in all his tissues. However, the qualitative and quantitative phenotypic expression of these polymorphisms may vary from tissue to tissue. Therefore it is necessary to be assured of the correlations between the phenotypings achieved from different tissues such as blood and semen.

Extensive testing of this correlation is limited by the availability of suitable semen samples accompanied by a corresponding blood or saliva sample or both. Over 250 corresponding blood and semen sample pairs (that is, 1 blood and 1 semen sample from each donor) have been examined and their correlation in ABO grouping, phosphoglucomutase (PGM_1) types and subtypes, and glyoxalase I (GLO I) types has been investigated. Amylase activities of most of the semen samples have also been measured.

Experimental Procedures

Grouping of blood samples in the ABO and Lewis systems was performed by classical serological techniques. Some of the blood samples were in a condition unsuitable for reliable Lewis grouping. A hemolysate of each blood sample (inclusive of those unsuitable for Lewis grouping) was prepared and stored at $-80°C$. All the semen samples were obtained as centrifuged cell-free plasma. These were also stored at $-80°C$.

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Semen samples were grouped in the ABO system by a manual absorption inhibition method [*1*] in microtitre trays. Four dilutions of semen, 10^{-1} to 10^{-4}, respectively, were tested.

Typing of blood and semen in the PGM_1 system was performed by starch gel electrophoresis [*2*].

Subtyping of PGM_1 isoenzymes was performed by isoelectric focusing (IEF) [*3*] on ultra thin (0.35-mm) acrylamide gels using pH 5.0 to 7.0 ampholytes (LKB) (supplied by Linbrook International Pty Limited, Adelaide, South Australia).

Typing of blood and semen in the GLO I system was performed by mixed agarose/starch electrophoresis [*4*].

Assays of semen samples for α-amylase activity were by spectrophotometric measurement of released dye from a chromogenic substrate (Amylochrome—Roche Diagnostica; supplied by Roche Products Pty Limited, Adelaide, South Australia).

Results

There were no qualitative contradictions when the groupings achieved from each of the semen samples were compared with the groupings achieved from their corresponding blood samples. However, the ability to conclusively group/type a semen sample varied and a number of inconclusive results were obtained in each of the ABO, PGM_1, and GLO I systems. This was probably due to quantitative variation in the genetically determined characters being examined or in vitro storage effects resulting from thawing and refreezing of samples or both. An unknown proportion of the semen samples were from infertile individuals and this could also account for variation between samples.

ABO Grouping

A blood sample providing an ABO group and a Lewis group of either Le(a−b+) or Le(a+b−) was necessary before the prediction of ABH antigenic substances in the corresponding semen sample could be attempted (165 samples) (Table 1). Since blood samples typing as Le(a−b−) or not being suitable for Lewis typing (114 samples) could not be used to determine secretor status, they are *not* included in Table 1.

The criterion for satisfactory correlation was based on the following. Agglutination of indicator cells at each dilution of semen was scored on a 0 (total inhibition) to 4+ (no inhibition) scale. This gave an aggregate score range of 16 points for samples with no inhibition in any of the four dilutions and 0 points for samples totally inhibited at all dilutions. An antigen was considered as being present if it achieved an inhibition score of 7 or less with the 10^{-1} dilution scoring either 0 or 1. An antigen was considered as being absent if it achieved an inhibition score of 11 or more with the 10^{-1} dilution scoring a minimum of 2. Inconclusive results were recorded for all aggregate scores of 8, 9, or 10 and for aggregate scores of 11 or more if the 10^{-1} dilution scored less than 2.

TABLE 1—*ABO grouping.*

	Blood Group A Le(a−b+)	Blood Group B Le(a−b+)	Blood Group O Le(a−b+)	Blood Group AB Le(a−b+)	Blood Groups A/B/O/AB Le(a+b−)	Total
No. of correlated blood/semen pairs	36	8	72	2	38	156
No. of pairs giving conclusive blood but inclusive semen results	2	1	3	...	3	9

TABLE 2—*Results of ABO grouping of nine semen giving inconclusive results.*

Blood Group	Aggregate of Inhibition Scores[a]		
	Anti-A	Anti-B	Anti-H[b]
O, Le(a−b+)	12	12	9
O, Le(a−b+)	14	16	9
O, Le(a−b+)	16	16	8
A, Le(a−b+)	11	15	6
A, Le(a−b+)	9	16	3
O, Le(a+b−)	8	14	11
O, Le(a+b−)	12	9	16
O, Le(a+b−)	10	8	14
B, Le(a−b+)	8	0	2

[a]Mean of three analyses.
[b]Saline extract of *Ulex europaeus.*

Using these criteria, 156 sample pairs showed satisfactory correlation (Table 1). Of these, 118 (or 75.6%) were classified as secretors and 38 (or 24.4%) as nonsecretors. However, semens from nine sample pairs gave inconclusive results (Table 2). Three of these were found not to contain their predicted H antigen in sufficient quantity to achieve an inhibition score of 7 or less (corresponding bloods typed as group O, Le[a−b+]). Two semen gave inhibition scores of less than 7 for H antigen but more than 7 for A antigen (corresponding bloods typed as group A, Le[a−b+]). The four remaining semen giving inconclusive results had inhibition scores of less than 11 but more than 7 for an antigen(s) not anticipated to be present. One sample had some A inhibition, one sample some B inhibition, and one sample some A and B inhibition (corresponding bloods all typed as Group O, Le[a+b−]). One sample had B, H, and some A inhibition (the corresponding blood typed as Group B, Le[a−b+]).

Of the 114 bloods for which secretor status could not be determined by Lewis grouping, it was possible to infer an ABO group for 87 of them (or 76.3%) from the groupings of their corresponding semen sample. No apparent contradictions to the ABO blood group were found. The remaining 27 semens (or 23.7%) giving inconclusive grouping results presumably arose from persons who were nonsecretors or secretors who provided samples containing quantities of ABH antigenic substances insufficient to be classified by the scoring system used.

PGM_1 Typing

Correlation between the blood hemolysate PGM_1 type and the PGM_1 type of the corresponding semen sample was demonstrated in 279 of the 283 tested pairs (Table 3). The observed banding intensities indicated that the level of seminal PGM_1 enzyme activity varied from sample to sample. However, no specific enzyme assays were performed to confirm this. This variation in activity probably accounted for three of the four samples giving inconclusive results as no seminal PGM_1 activity was observed at all (Table 3). The fourth sample gave a blood PGM_1 1. Its corresponding semen PGM_1 type was of only moderate overall activity but whose isoenzyme banding pattern (two bands observed) could not unequivocally be identified as being either PGM_1 1 or PGM_1 2−1. It was therefore regarded as inconclusive. Treatment of this semen with reducing reagents did not alter the banding pattern. The same pair of samples gave a satisfactory correlation of PGM_1 1+1− by IEF.

One correlated pair of samples from an Oriental male was nominally typed[2] as a PGM_1 6−2 (Fig. 1).

[2]Results confirmed by Dr. R. L. Kirk, Department of Human Biology, John Curtin School of Medicine, Canberra, Australia.

TABLE 3—*PGM_1 typing.*

	PGM_1 1	PGM_1 2−1	PGM_1 2	Total
No. of correlated blood/semen pairs[a]	155	104	19	278
No. of pairs giving conclusive blood but inconclusive semen results	2	2	...	4

[a]Rare phenotype PGM_1 6−2 not included in table.

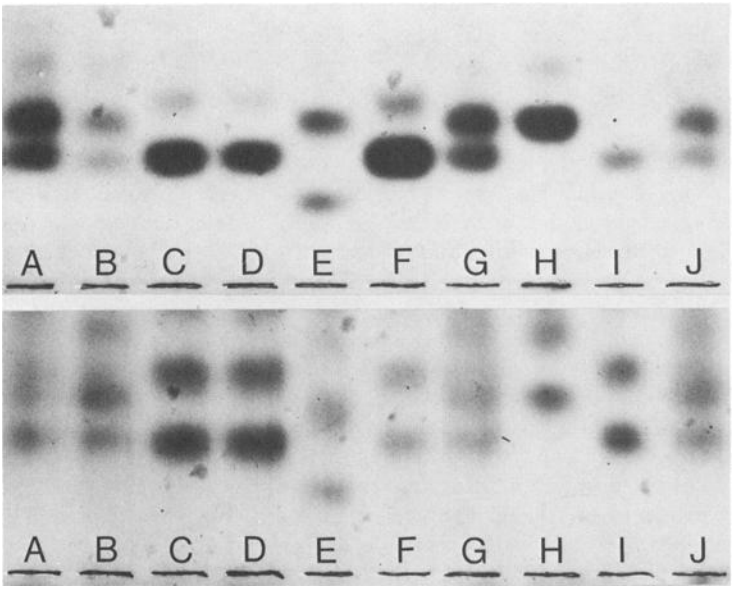

FIG. 1—*Correlation of PGM_1 types in "paired" blood and semen samples. Upper row are semens, lower row are hemolysates. The paired samples* A, B, G, *and* J *typed as PGM_1 2−1; samples* C, D, F, *and* I *typed as PGM_1 1; and samples* H *typed as PGM_1 2. The pair of samples* E *were nominally typed as PGM_1 6−2.*

PGM_1 Subtyping (IEF)

Correlation between the blood hemolysate PGM_1 subtype and the PGM_1 subtype of the corresponding paired semen sample was demonstrated in 267 of the 280 tested pairs (Table 4). Thirteen sample pairs gave inconclusive results (Table 4). Two of these (hemolysates PGM_1 2−1 [2−1−] and PGM_1 1 [1+1−]) showed no seminal PGM_1 activity on either starch or IEF gels. The remaining eleven samples (five hemolysates PGM_1 1+1−, two PGM_1 1+, three PGM_1 2+1+, and one PGM_1 2−1+) all gave a correlated blood/semen pair result by starch electrophoresis but failed to show any seminal PGM_1 activity by IEF. All eleven samples had semen PGM_1 activity levels assessed on starch electrophoresis to be weak or very weak.

One correlated pair of samples from an Oriental male was nominally typed[3] as PGM_1 6,2− (Fig. 2).

[3]Results confirmed by Dr. R. L. Kirk, Department of Human Biology, John Curtin School of Medicine, Canberra, Australia.

TABLE 4—*PGM_1 subtyping.*

	1+	1+1−	1−	2+	2+2−	2−	2+1+	2−1+	2+1−	2−1−	Total
No. of correlated blood/semen pairs[a]	102	43	4	11	7	1	63	17	17	1	266
No. of pairs giving conclusive blood but inclusive semen results	2	6	...	...	...	...	3	1	...	1	13

[a] Rare phenotype PGM_1 6,2− not included in table.

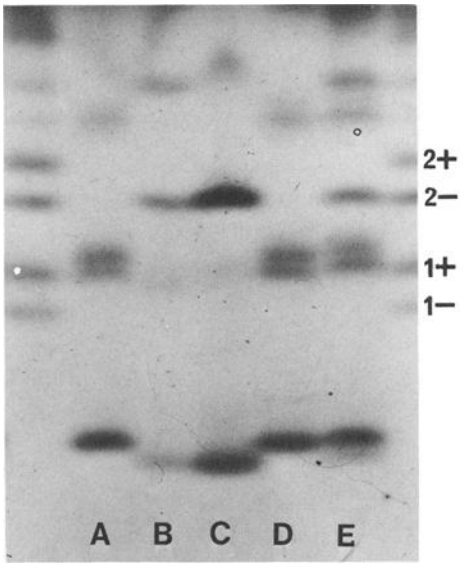

FIG. 2—*Correlation of PGM_1 subtypes in blood and semen. The blood sample nominally typing as PGM_1 6,2− (B) may be compared with its paired semen sample (C). The same figure also shows the subtyping of another rare variant hemolysate (both A and D). This has been nominally called PGM_1 1+ Adelaide. A mixture of hemolysates PGM_1 6,2− and PGM_1 1+ Adelaide shows their cathodal PGM_1 bands to be clearly separable (E).*

GLO I Typing

Correlation between the blood hemolysate GLO I type and the GLO I type of the corresponding semen sample was demonstrated in 255 of the 271 tested pairs (Table 5). The observed banding intensities indicated that the level of GLO I enzyme activity varied from sample to sample. However, no specific enzyme assays were performed to confirm this. Sixteen sample pairs gave inconclusive results (Table 5). Eleven of these (four hemolysates GLO 1, five GLO 2−1, and two GLO 2) showed no seminal GLO I activity and one (hemolysate GLO 2−1) showed only weak activity. The remaining four sample pairs (one hemolysate GLO 1, one GLO 2−1, and two GLO 2) gave very smeared patterns of seminal GLO activity of similar mobility to their corresponding hemolysates. However, no unequivocal GLO I typing could be made.

TABLE 5—*GLO I typing.*

	GLO 1	GLO 2−1	GLO 2	Total
No. of correlated blood/semen pairs	51	123	81	255
No. of pairs giving conclusive blood but inconclusive semen results	5	7	4	16

Semen Amylase Determinations

The semen amylase activities of 214 semen samples were measured. Their frequency distribution is depicted in Fig. 3. Activities appeared to fall into two groups. It was calculated that the mean amylase activity of the major group (203 samples) was 77 IU/L (range 10 to 498 IU/L, standard deviation 80 IU/L). The remaining eleven samples had a high to extremely high amylase activity (range 699 to 4440 IU/L).

Discussion

All testing was performed on liquid samples and not stains prepared from the liquid samples. This was because the purpose of testing each sample was to examine the correlation between the (original) biological fluids. The results are therefore in no way to be taken as implying the same correlation necessarily exists between a semen-containing stain or swab in a casework

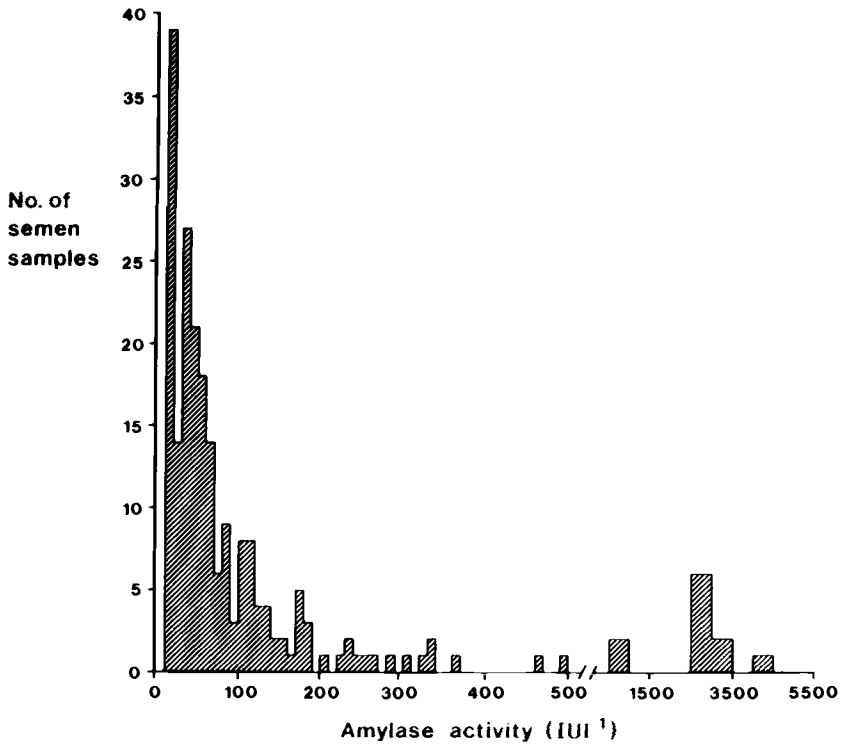

FIG. 3—*The frequency distribution of α-amylase activities in 214 semen samples.*

example and blood from the correct suspect. For this correlation to be satisfied, an additional assumption is required. This is that the semen in the stain does not alter or lose its original grouping character during its drying process, its admixture with other biological fluids, or its aging as a stain. It was not the purpose of these tests to examine this assumption, though it has been reported semen may apparently undergo changes in stains of mixed biological fluids [*5,6*].

Since the discovery of ABH antigenic substances in semen [*7*], neither the extensive investigations concerning the origin of their presence on the sperm cell surface [*8–10*] nor the reported polymorphism [*11*] of ABH antigenic substances in seminal plasma has had as significant a practical influence on their forensic science analysis as that information concerning their quantity and proportion in seminal plasma. This has been obtained by both manual [*12*] and automated methods [*13,14*]. Using the stated criteria for interpretation, the results of the present study demonstrate that a minority of semens may prove difficult to group reliably. Nine such semens were so categorized in this study (Table 2). Two of these, had they been encountered in casework, may, by an inhibition method of analysis alone, have been mistyped as coming from a Group O secretor (H antigen) when in fact their source was a Group A secretor (both A and H antigens) (Table 2). Three semens appeared to contain only small amounts of H antigen and failed to conclusively demonstrate their source was in fact a Group O secretor (Table 2). These sorts of difficulties have been previously described [*13,14*] and their relevance to casework samples considered [*15,16*]. Interpretation of ABO groupings arising from mixtures of both seminal secretions and vaginal material is additionally complicated by similar quantitative variation of ABH antigenic substances in vaginal material [*17*].

The blood typings of Le(a+b−) and Le(a−b+) have been used to describe nonsecretors and secretors [*13,14*]. It is now understood, however, that both the quantity and ratios of ABH antigenic substances present in individuals in any population is distributed in a broad continuum [*14*]. Some individuals whose blood types as Le(a+b−) have sufficient H substance to be detected by an automated inhibition technique [*13*]. Replicate analyses of three semen samples in this survey, anticipated to contain no ABH antigenic substances as their corresponding bloods typed as Group O, Le(a+b−), showed some slight and inconclusive inhibition by apparent A and B antigenic substances but not H antigen (Table 2). There were no duplicate semen samples available from these individuals and the reasons for these findings are unknown. Generally the use of the terms secretor and nonsecretor remain appropriate. However, recent research shows the secretor system to be extremely complex. For example, the glycosyltransferase enzymes conferring A, B, H, and Le specificity are not only structurally distinct [*18*], but they may also be of differing efficiency in competition for their substrate sites on both the precursor oligosaccharide chain or on H substance itself [*19,20*].

None of the semens in this study were typed for Lewis substances and although possible [*21,22*], it does not appear to be performed routinely in many forensic science laboratories.[4] The greater specificity of monoclonal Lewis antibodies [*23*], were these to become widely available, will hopefully assist the reliable reporting of ABH antigenic substances in both saliva and semen.

The correlation between blood PGM_1 type and semen PGM_1 type is well established [*6,24–30*]. Two apparent anomalies in the correlation between blood and semen PGM_1 were reported [*6*], but these were not from direct comparison of original body fluids but rather semen recovered from the vagina of the consenting partner. In another survey [*29*] a further 2 anomalies were reported from an examination of 50 blood/semen pairs. One of these appeared to be the result of preferential loss of activity of the product of the PGM_1^1 allele in comparison to that from the PGM_1^2 allele. The other was restored to a recognizable and correlated PGM_1 2−1 type after treatment with a reducing reagent. A survey of 107 semens [*30*] (for which no corresponding bloods were available) from vasectomized males produced 5 uninterpretable results,

[4]Personal communication, Mr. A. M. Ross, Forensic Biology Laboratory, Adelaide, Australia, Nov. 1983.

all of which were transformed to recognizable PGM_1 phenotypes on treatment with a reducing reagent.

The semen samples in the present survey, none of which were from vasectomized males, were generally run in the absence of reducing reagents. However, a random selection of approximately 80 semen were run in duplicate, with and without reducing agents. None appeared to be influenced by this treatment, including the one sample that could not be unequivocally identified (its corresponding blood typed as PGM_1 1). Thus the usefulness of such treatment of semen before PGM_1 typing appears to be unconfirmed. This is apparently also true for PGM_1 analysis of seminal stains in casework where reducing reagents are not routinely used.[5] The small number of semen samples showing no detectable PGM_1 activity after starch gel electrophoresis (about 1%) is of similar order to those reported in previous surveys [*6,29,30*] and is also consistent with the reported variation of PGM_1 activity measured in seminal plasma [*31,32*] and sperm [*32*]. The possibility that the variation in activity seen in this survey is in part attributable to the fertility of the donor cannot be excluded.

The PGM_1 subtyping by IEF of both seminal plasma [*33*] and sperm lysates [*34*] has been reported, the phenotypic frequency distribution from semen samples being in close agreement with that from blood [*33*]. Correlation between 95 paired blood/semen samples has also been reported [*35*] with no anomalies being established. No anomalies were found in this present survey but 13 samples gave inconclusive results as they failed to demonstrate any activity on IEF.

The enzyme activities of PGM_1 subtypes in blood have been reported [*36*] and the PGM_1 1+1− phenotype is claimed as the least active.[6] The total bloods in this survey subtyping as PGM_1 1+1− (49 samples) comprised approximately 17% of all the bloods subtyped in this study (280 samples) (Table 4). However, a large number of their paired semen (6 of the 49 samples or 12%) gave inconclusive results (Table 4) as they displayed no activity on the IEF gel. This rate of failure was proportionately greater than for the other PGM_1 subtypings (apart from PGM_1 2−1− where one of only two sample pairs gave conclusive results). This suggests that the activities of seminal PGM_1 isoenzymes may be distributed in a manner similar to blood but at an overall lower level of activity [*32*]. If this phenotypic activity distribution exists, it may best be evident in the 10 common IEF subtypes of PGM_1 as it appeared not to be present in a survey of 150 samples in which total semen PGM_1 activity was assayed and classified according to the three common PGM_1 phenotypes [*31*].

In general, the PGM_1 results from IEF of semen samples differ from those of blood in that the products of the PGM_2 and PGM_3 locus are absent and the intensity of the banding attributable to the secondary isoenzymes from the PGM_1 locus are less than blood. The concern that a semen known to come from a male who is genetically heterozygous for PGM_1 but whose seminal PGM_1 displays activity at only one IEF band position but not the other because of disproportionate levels of enzyme activity did not materialize in the samples examined. However, it is still regarded as a potential hazard, and probably more so in stain analysis.

An uncommon variant, nominally typed as PGM_1 6,2−, was found in both the blood and semen of an Oriental male (Fig. 2). Since the hemolysate PGM_1 subtyping of PGM_1^7 and PGM_1^3 each into two subtypes has been reported [*37,38*], it is speculated that the PGM_1^6 may eventually be similarly subtyped, although 17 PGM_1^6 samples failed to show this [*38*].

The correlation between the common GLO I phenotypes and semen GLO I phenotypes has been reported [*39,40*]. To date no apparent anomalies have been reported and this survey located none. However, the multiple secondary anodic banding evident in all phenotypes in both blood and semen samples meant that results had to be carefully interpreted from the primary GLO I banding. This multiple secondary banding lead in some cases to poorly defined smeared results. The general lability of the enzyme, particularly in semen, was probably the reason for the large number of semen samples for which inconclusive results were obtained (16 samples

[5]Personal communication, Dr. T. J. Rothwell, Home Office, London, England, Oct. 1983.

[6]Gene activity order: $PGM_1^{1-} < PGM_1^{1+} = PGM_1^{2-} < PGM_1^{2+}$. Phenotype activity order: PGM_1 1+1− < 1+ = 2− = 2−1− = 2+2− = 2−1+ = 2+1− < 2+ = 2+1+ (1− not tested).

out of 271). No obvious explanation could be advanced as to why the correlation failure rate was apparently greater for GLO 1 than either GLO 2−1 or GLO 2 (Table 5). The GLO I enzyme is apparently particularly labile when mixed with vaginal secretions [*41*] and for this reason GLO I typing is likely to remain confined to seminal stains uncontaminated with other body fluids.

The range of semen amylase activity levels found in this survey is similar to those previously reported [*42,43*]. The semen in this survey did not include any samples from vasectomized individuals but they did include some semen from subfertile individuals. The most interesting feature arising from this survey is the small number of samples (approximately 5%) with abnormally high amylase levels. This was also consistent with previous results [*43*]. No corresponding saliva samples were available for testing and therefore no comparison with salivary amylase levels could be made. However, one explanation for the abnormally high amylase levels in some semen could be contamination (possibly with saliva) during collection. The implication of these results is that the finding of high amylase levels in a seminal strain may not in every case indicate its contamination with saliva.

Acknowledgments

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