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J. C. Standefer,[1] *Ph.D. and E. W. Street,*[1] *B.A.*

Postmortem Stability of Prostatic Acid Phosphatase

Elevated prostatic acid phosphatase activities in samples collected from the vaginal area are commonly accepted as presumptive evidence for the presence of seminal fluid, and this enzyme activity has been used extensively as medicolegal evidence of carnal knowledge. The forensic significance of the acid phosphatase determination has increased as the number of vasectomized males has increased, since a positive acid phosphatase assay may be the only indication of the presence of seminal fluid when sperm are absent. If the acid phosphatase determination can contribute significantly to a forensic investigation of rape/murder cases it is imperative that the investigator make an accurate interpretation of the enzyme assay. The accuracy of this interpretation is in part related to the postmortem stability of acid phosphatase, both in situ and during specimen storage.

The survival time of acid phosphatase in the vagina of living persons following intercourse has been established at about 24 to 36 h [*1*]. Postmortem survival is less well defined but has been reported to be between 32 h and 16 days [*2,3*]. This study is designed to provide two types of information which will be helpful for interpretation of acid phosphatase values in samples collected at autopsy. First, stability of acid phosphatase after collection was assessed by analyzing body fluids supplemented with seminal fluid and stored under various conditions. These data provide optimum storage conditions for collected samples and indicate the maximum time the sample may be stored before significant activity loss occurs. Second, in-situ stability of acid phosphatase was evaluated by assaying fluids taken at autopsy of rape/murder victims who had been dead for various periods prior to autopsy. Since the analyzed fluids had remained in situ during the postmortem interval, we conclude that acid phosphatase may remain active in the vaginal area at least 7 days after death, in the rectal area 24 h after death, and in the oral cavity 36 h after death. All of these times may be extended up to one month if the body remains at low temperatures.

Materials and Methods

Reagents

To prepare citrate buffer, 0.1*M*, pH 6.0, dissolve 14.7 g sodium citrate and 0.8 sodium chloride in 450 ml water, add 5 g Brij-35 (Technicon Inc.), and adjust pH to 6.0 and volume to 500 ml. This buffer is stable for three months at 25 °C.

To prepare the color reagent, dissolve 0.75 g Naphthanil diazo blue B and 0.5 g Brij-35 in 100 ml deionized water. This reagent is stable at 4 °C for at least 3 months.

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[1] Toxicologists, Office of the Medical Investigator, University of New Mexico School of Medicine, Albuquerque, N. Mex. 87106

To prepare the substrate, dissolve 0.335 g α-naphthol phosphate in 25 ml distilled water. This substrate is stable at 4 °C for 6 weeks.

Method

The procedure for acid phosphatase determination was modified from that reported by Babson et al [*4,5*]. All reagents were brought to room temperature; then saline from the specimen container (0.1 ml), buffer (1.7 ml), and color reagent (0.1 ml) were mixed in a 10 by 75-mm disposable glass test tube. The absorbance at 520 nm was recorded in a 1-cm cuvette, substrate (0.1 ml) was added, and the absorbance was again recorded at 1-min intervals for 5 min. An average absorbance change per minute was calculated, and this change in absorbance per minute is equivalent to mIU of acid phosphatase activity. The relationship between absorbance and mIU was determined by substituting prepared solutions of α-naphthol substrate in the reaction.

Preparation of Acid Phosphatase Pools for In-Vitro Study

Swabs were used to collect fluids from the rectal, vaginal, and oral cavities at autopsy of individuals with no history of recent sexual experience. The autopsy was conducted and the swabs were collected within 24 h of death. The swabs were placed in test tubes, and 1 ml of 0.85% saline was added. After overnight storage at 4 °C, the saline from the tubes was collected into oral, rectal, and vaginal pools and these pools were supplemented with seminal fluid to produce a final acid phosphatase activity of approximately 500 mIU in each pool. Aliquots of these pools were stored at either 25, 4, or −20 °C and analyzed for acid phosphatase activity after various intervals of storage.

Collection of Samples at Autopsy

Two cotton-tipped, 20-cm swabs are used to wipe the area of interest and then placed together in a plastic, screw-capped test tube. One cubic centimetre of 0.85% saline is placed in the tube, and the swab tips are massaged to liberate collected material into the saline. The tubes are then stored at 4 °C. The saline solution is analyzed for acid phosphatase activity within 24 to 48 h.

Results

Assay Method

In the assay the substrate concentration is 5 m*M*, which supports zero order kinetics and linear reaction rates over a wide range of acid phosphatase activities (Fig. 1). The effect of adding oral or rectal fluids to solutions of seminal fluid is minimal, that is, less than 5% decrease in reaction rate (Fig. 2). Also, adding whole blood which was hemolyzed by 1:1 dilution with distilled water does not significantly affect the reaction rate (Fig. 3). The absorbance at zero time is increased, but hemolyzed blood alone has little acid phosphatase activity in this system and the reaction rate is not altered by adding increments of hemolyzed blood to the reaction mixture. When various dilutions of a topical contraceptive were added to the reaction mix, there was an increase in absorbance during the initial 15 s of the reaction but none during the subsequent 5-min interval.

The upper limit for determination of a negative reaction was established by analyzing swabs taken at autopsy from persons with no known recent sexual experience. If the value of this upper limit is taken as greater than three standard deviations from the mean of the assays, the upper limit for a negative assay would be 30 to 50 mIU (Table 1),

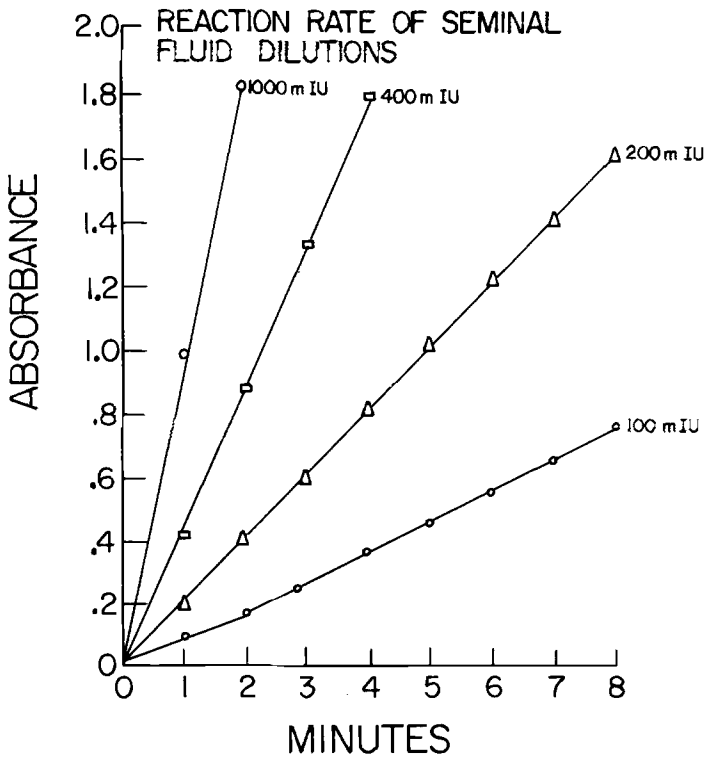

FIG. 1—*Linear reaction rates: aliquots of diluted seminal fluid are added to the reaction mixture and the total absorbance change is monitored. The reaction rates are linear for acid phosphatase activities up to 1000 mIU.*

depending on the source of the assayed fluid. The precision of the assay was determined at three levels of acid phosphatase activity and at the critical borderline level is approximately ±3% (Table 2).

In-Vitro Stability of Acid Phosphatase

The aliquots of saline supplemented with seminal fluid were analyzed at 1, 2, 5, 15, 30, 60, and 200 days after preparation, and the results are shown for oral fluid (Fig. 4), rectal fluid (Fig. 5), and vaginal fluid (Fig. 6). Generally, the fluids retained a significant portion of their acid phosphatase activity for 30 to 60 days when stored at 4 or −20°C. At room temperature all fluids lost considerable activity, and the rectal fluid had no detectable activity after 5 to 10 days at 25°C.

In-Situ Stability of Acid Phosphatase

Swabs taken at autopsy of cases in which there were well-documented histories of sexual abuse were collected and analyzed as described. There is evidence that significant acid phosphatase activity can be detected in vaginal fluids 7 days postmortem, in oral fluids 36 h postmortem, and in rectal fluids 24 h postmortem (Table 3). One case is listed in which significant acid phosphatase was measured in the three fluids 30 days postmortem. This individual was discovered frozen in a snow-covered, shallow grave and the temperature at this site ranged from −20 to −10°C during the postmortem period.

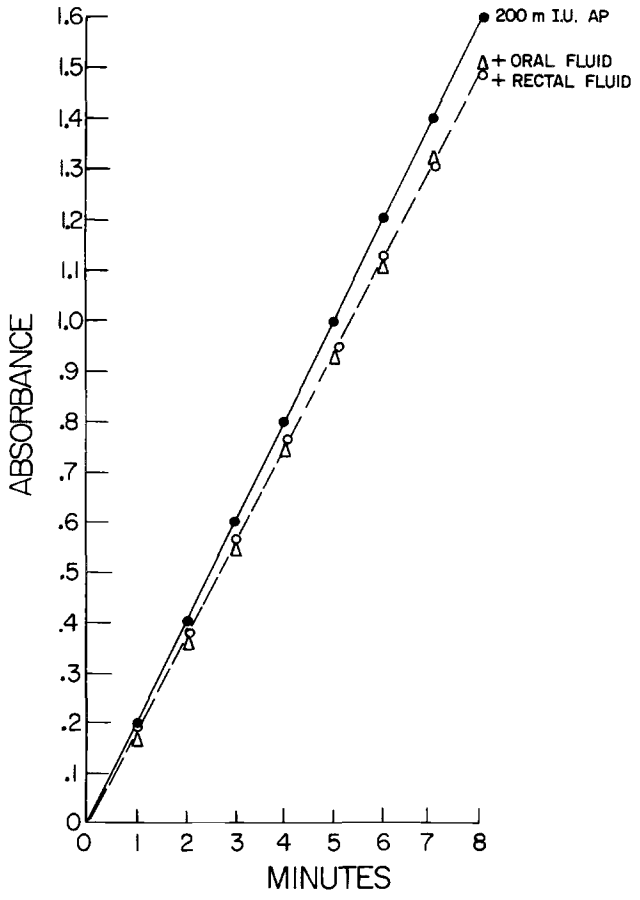

FIG. 2—*Effect of oral and rectal fluid on reaction rates: oral and rectal fluids negative for prostatic acid phosphatase were collected at autopsy and added to a reaction mixture containing 200 mIU prostatic acid phosphatase.*

Discussion

The method used in this study is modified from that described by Babson and Read [*4*] to permit kinetic measurements of the acid phosphatase activity. The kinetic assay provides for recording multiple data points during the reaction which can be averaged to increase the precision. The kinetic assay also obviates the need for the simultaneous blank determination. We observed no significant effect on the assay of potential enzyme inhibitors which may be present in rectal or oral fluids, and since the kinetic assay accounts for nonspecific, rapid color development which can be caused by topical contraceptives [*6*], we conclude that the assay is suited for accurate acid phosphatase determinations in oral, rectal, or vaginal fluids. The lack of significant change in the reaction rate when hemolyzed blood is added indicates that the red blood cell acid phosphatase has low reactivity in this system and therefore bloody specimens would not obviate an accurate assay. However, one should use caution in analyzing bloody rectal swabs. If the blood in the rectal area is a result of severe internal trauma, prostatic acid phosphatase could be contributed directly by a ruptured prostate.

The lower limit of activity above which the assay in vaginal fluids is considered positive is taken as 50 mIU. This allows for a +3 standard deviation increment from the mean of

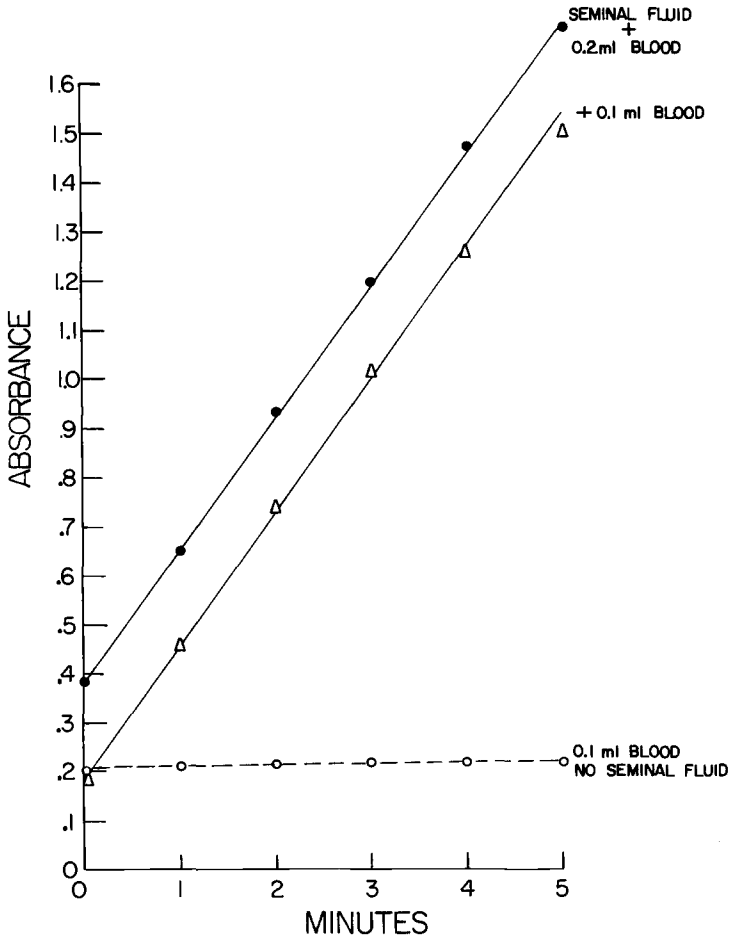

FIG. 3—*Effect of hemolyzed blood on reaction rates: whole blood was hemolyzed by adding an equal volume of water to packed red blood cells. The hemolyzed mixture was added to the reaction mixture, either alone or with 250 mIU of prostatic acid phosphatase.*

TABLE 1—*Levels of acid phosphatase in controls.*

Fluid	Samples, no.	Avg Level, mIU	3-SD Range
Oral	30	12	0–40
Rectal	30	10	0–31
Vaginal	30	18	0–48

the 30 negative samples and an additional ±4% to accommodate the imprecision of the assay (±3%) at that level of acid phosphatase activity. For simplicity and to allow an extra measure of caution in the interpretation of the acid phosphatase, we have chosen 50 mIU as the lower cut-off value for positive determinations in oral and rectal fluids.

Postmortem identification of sperm has been documented in vaginal fluids 16 days

TABLE 2—*Precision of acid phosphatase assay*

Activity	Samples, no.	Mean	Standard Deviation	Coefficient of Variation, %
Low	6	9.3	± 0.82	± 8.8
Borderline	6	61.2	± 1.9	± 3.1
High	10	279	± 6.0	± 2.1

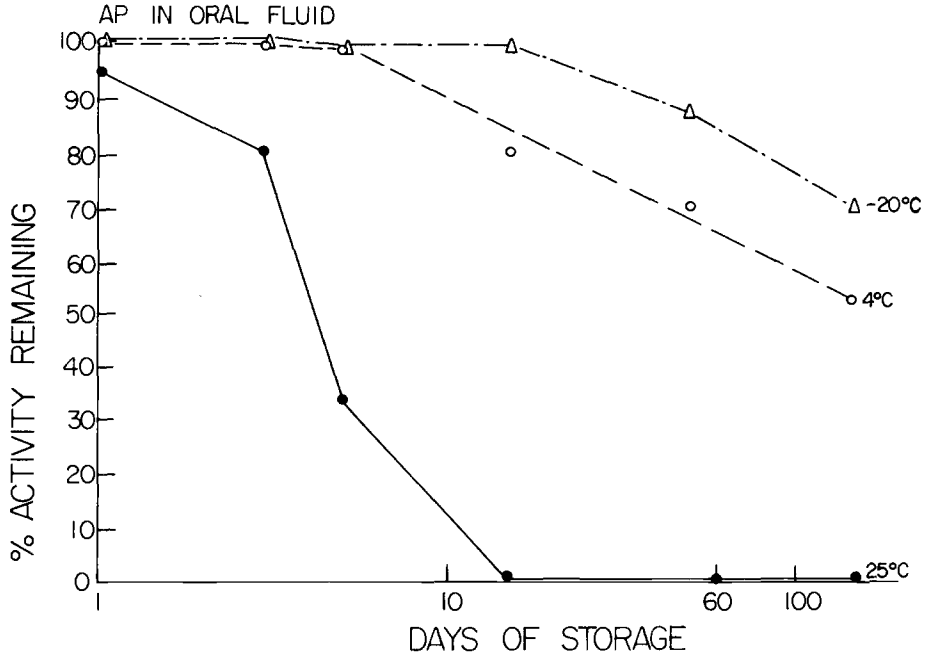

FIG. 4—*Stability of prostatic acid phosphatase from oral fluids during storage: oral fluids negative for acid phosphatase were collected at autopsy, supplemented with seminal fluid, and analyzed after periods of storage at −20, 4, and 25°C.*

after death [*2*], however, no acid phosphatase data were presented. Our study indicates that acid phosphatase activity could be detected up to one month postmortem in vaginal areas. Although no conclusive data are available, the enzyme is probably more labile in the rectal and oral areas. The supposition that the acid phosphatase would be less stable in these fluids is supported by increased lability in these fluids observed during in-vitro storage. Our results indicate that one can observe prostatic acid phosphatase in the rectal and oral areas up to 24 to 36 h postmortem and that this survival time can be extended when the body remains at low temperatures during the postmortem interval.

Appropriate storage conditions after collection of the specimens are necessary for accurate prostatic acid phosphatase determinations, particularly in oral and rectal fluids. One should not expect to find significant amounts of acid phosphatase in rectal or oral fluids after 5 to 10 days storage at 25°C. If these types of specimens must be sent to other laboratories for acid phosphatase assay, they should be shipped frozen. At −20°C the fluids will retain significant activity for at least two months.

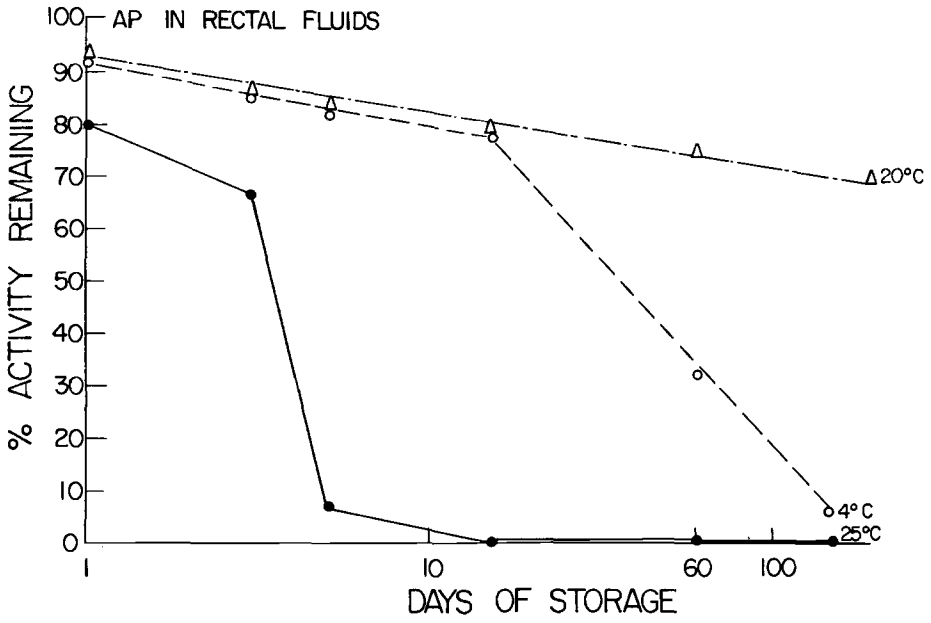

FIG. 5—*Stability of prostatic acid phosphatase from rectal fluids during storage: rectal fluids negative for acid phosphatase were collected at autopsy, supplemented with seminal fluid, and analyzed after periods of storage at −20, 4, and 25°C.*

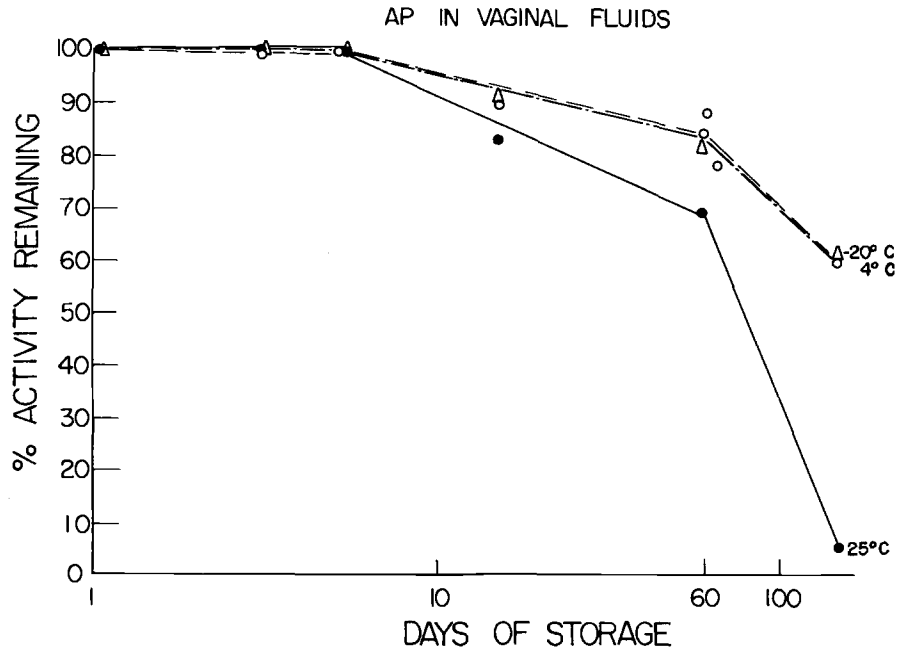

FIG. 6—*Stability of prostatic acid phosphatase in vaginal fluids during storage; vaginal fluids negative for acid phosphatase were collected at autopsy, supplemented with seminal fluid, and analyzed after periods of storage at −20, 4, and 25°C.*

TABLE 3—*In-situ postmortem stability of acid phosphatase.*

Case	Swabbed Area	Level, mIU	Sperm	Postmortem Interval
2254	vagina	122	+	24 h
1358	vagina	60	+	36 h
2398	vagina	210	–	5 days
2608	vagina	900	+	6 days
2577	vagina	80	–	8 days
7001	vagina	3000	+	30 days[a]
2254	oral cavity	230	+	24 h
1358	oral cavity	100	+	36 h
7001	oral cavity	2500	+	30 days[a]
2251	rectum	222	+	12 h
2254	rectum	80	+	24 h
7001	rectum	730	+	30 days[a]

[a] Postmortem interval was at −20 to −10°C.

Conclusions

1. Prostatic acid phosphatase is accurately and precisely measured by a kinetic procedure using α-naphthol phosphate as the substrate.
2. Samples collected at autopsy should be analyzed within 48 h after collection and should be stored at 4°C.
3. Storage of specimens for up to two months is possible if they are kept at −20°C.
4. One can expect to observe positive prostatic acid phosphatase activity in the vagina up to 7 days, in the oral area 36 h, and in the rectum 24 h postmortem.
5. In-situ survival of acid phosphatase is temperature dependent, and the postmortem survival time can be greatly extended if the body remains at low temperature.

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Office of the Medical Investigator
University of New Mexico School of Medicine
Albuquerque, N. Mex. 87106