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## In Vitro Studies on a Breathalyzer

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The Breathalyzer is an instrument designed and developed by Borkenstein<sup>2</sup> for use by law enforcement agencies for rapid, accurate, convenient, and positive recognition of alcoholic intoxication as defined by law. Because the use of the Breathalyzer as a means for determining the alcohol (ethanol) concentration of the blood has recently increased, the judge of a Justice Court in Spokane, Washington desired to know more about the reliability of the instrument. A Breathalyzer used by the Washington State Patrol in the Spokane area was provided for a detailed investigation. This instrument (Model 100) was examined and operated after delivery under a variety of experimental conditions to determine any errors or inconsistencies in its performance. The investigation involved only the Breathalyzer and its accessories and did not include tests of human subjects or physiological tests.

Coldwell and Grant<sup>3</sup> described a number of tests on the Breathalyzer. Some of the features adequately covered were concentrations of the dichromate solution and the sulfuric acid, temperature of the ampule, amount of solution, and instrument response over a range of alcohol concentrations; thus, these tests were not duplicated in the present investigation. The scope of this investigation covered the photoelectric system, chemical reaction, aging of ampules, bubbler orifice size, absorption of ethanol in the tubing, condensation, alcohol vapor in the room, voltage variation, and effect of bright lights.

### Experimental

A solution was prepared containing 0.025 percent each of potassium dichromate and silver nitrate in 50 percent by volume sulfuric acid in water. A solution of 0.40 percent weight per volume of ethanol in water was also prepared. (Concentrations are expressed in percent weight per volume except where otherwise noted.) These solutions were used in several experiments; in others, commercial ampules prepared by the Stephenson Co. were used.

A breath sample simulator was prepared by attaching a rubber squeeze bulb to a sintered glass diffusion finger and inserting the finger into a bottle of 0.40 percent ethanol in water. The bottle was sealed with a stopper through which passed the tube from the squeeze bulb, the outlet tube, and a thermometer. Air that was pumped through the solution with the squeeze bulb became saturated with water and alcohol vapor at the temperature of the solution. The vapor passed from the head space in the bottle through an exit tube, which could then be attached to the Breathalyzer. The temperature of the solution was read when a sample was taken to determine the expected concentration of ethanol vapor.

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<sup>2</sup> Borkenstein, R. F. and Amith, H. W., *Medicine, Science and the Law*, MDSLA, Vol. 1, 1961, pp. 13-21.

<sup>3</sup> Coldwell, B. B. and Grant, G. L., *Journal of Forensic Sciences*, JFSCA, Vol. 8, 1963, pp. 149-162.

This vapor sampler was used for some experiments to simulate breath from a subject with a blood alcohol concentration (%BA) of approximately 0.16. Direct injection of the 0.40 percent ethanol was used in other experiments where it was desired to eliminate any variables caused by the vapor sampling system. Mixing was accomplished by turbulence from the injection and a final swirling of the ampule before insertion into the instrument. Since these tests concern the Breathalyzer only and not human subjects, all values of %BA refer to dial pointer readings of the instrument and not actual blood alcohol concentrations.

A. *Reliability of Photoelectric System*—The instrument was set up according to the procedure recommended by the inventor,<sup>2,4</sup> and microliter ( $\mu$ l) quantities of the 0.40 percent ethanol were injected into sample ampule. The instrument dial pointer reading was noted. Then the pointer was moved and the instrument was brought to balance again. This was repeated for a total of 20 readings on the same sample without moving the ampule. The data are given in Table 1.

TABLE 1—Dial pointer reading for several ethanol concentrations.

0.40% Ethanol, $\mu$ l	Dial Pointer Reading, %BA	Standard Deviation
5	0.094	$\pm 0.003$
10	0.168	$\pm 0.008$
15	0.263	$\pm 0.004$

B. *Linearity of Photoelectric and Mechanical Systems*—The instrument dial pointer was zeroed, and without introducing any sample the pointer was moved successively to each mark on the scale by adjustment of the light position. The current produced by the unbalanced photocells was read with a separate meter. The current rose linearly at the rate of 0.30  $\mu$ A per scale division (0.01 percent).

C. *Chemical Reaction*—The instrument was operated according to standard procedure except that liquid samples of ethanol in water (0.40 percent) were injected directly into the dichromate solution. The results for eleven trials each are given in Table 2 and Fig. 1.

TABLE 2—Results of eleven injection trials.

Amount Injected, $\mu$ l	Dial Pointer Reading, %BA	Standard Deviation
5	0.086	$\pm 0.002$
7	0.122	$\pm 0.005$
10	0.169	$\pm 0.004$
15	0.250	$\pm 0.004$
20	0.308	$\pm 0.005$

D. *Change in Reading with Time*—The instrument was operated according to standard procedure except that the readings were made at several times after the normal 1.5-min wait after sample introduction. The results for three trials are shown in Fig. 2.

E. *Heat Aging of Ampules*—The instrument was operated according to standard procedure using injected samples of ethanol. Commercial ampules were kept in an oven at 110 C for the periods indicated in Table 3.

<sup>4</sup> Whitman, DeWitt, Breathalyzer Training Manual, Washington State Patrol, 1963.

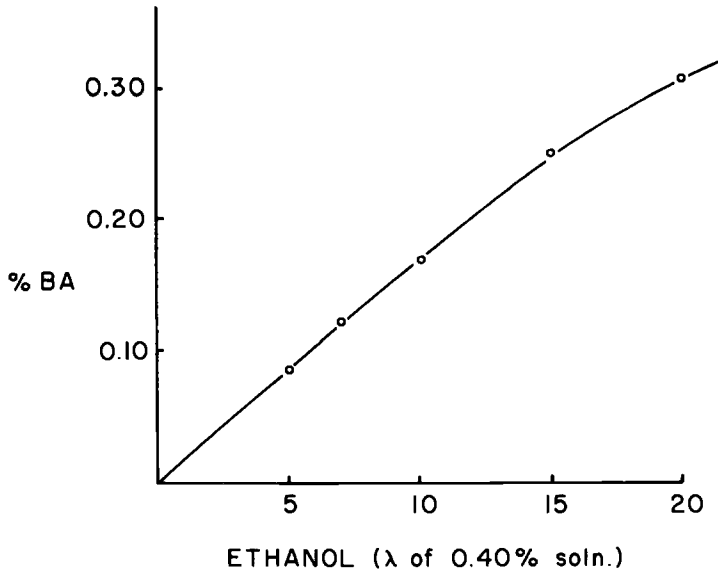


FIG. 1—Breathalyzer response to injected ethanol.

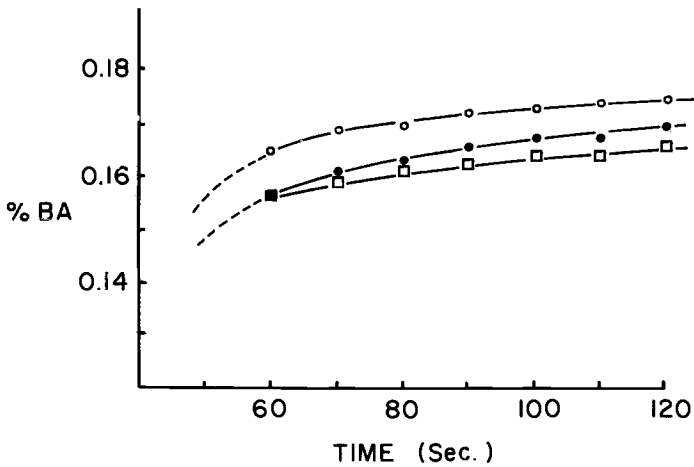


FIG. 2—Change in reading with time.

TABLE 3—Oven time for ampules.

Time in Oven, h	Number of Trials	Dial Pointer Reading, %BA	Standard Deviation
0	10	0.187	±0.008
24	9	0.183	±0.004
64	10	0.180	±0.003

F. *Variation in Bubbler Orifice*—Commercial glass bubbler tubes were modified to have a larger and a smaller orifice than normal. The instrument was operated according to standard procedure using dilutious of 0.40 percent ethanol in the breath sample simulator. The results of 20 trials each using the modified and one unmodified orifice are shown in Table 4.

TABLE 4—*Effects of bubbler orifice variation.*

Orifice Diameter, mm	Bubbling Time, s	Dial Pointer Reading, %BA	Standard Deviation
2.21	14	0.168	±0.006
1.17 (unmodified) <sup>a</sup>	14	0.168	±0.004
0.31	27	0.175	±0.005

<sup>a</sup> The measurement of nine commercial bubblers showed an average orifice diameter of 1.16 mm.

G. *Retention of Ethanol in the System*—The instrument was operated according to standard procedure using vapors from the 0.40 percent ethanol solutions in the breath sample simulator as the initial sample. These samples were vented to the room without passing through the ampule. After a 5-min wait, the cylinder was flushed with 20 pulses of room air, and the air in the cylinder was then bubbled through the ampule. The reading was noted and the pointer was reset at zero. The cylinder was charged with room air again and then discharged through the ampule. Another reading was taken, and the process was repeated several times. The results are given in Table 5.

TABLE 5—*Retention of ethanol after flushing.*

Tubing	Length, cm	No. of Trials	Dial Pointer Reading, %BA for avg of indicated number of trials							
			1st	2nd	3rd	4th	5th	6th	7th	8th
Original <sup>a</sup>	1.2	3	0.033	0.010	0.003	0.004	0.006	0.003	0.004	0.002
New <sup>c</sup>	1.4	1	0.048	0.016	0.000	0.001	0.001	...	...	...
New <sup>a</sup>	5.9	3	0.029	0.006	0.009	0.002	0.001	0.003	0.001	...
New <sup>b</sup>	5.7	3	0.006	0.016	0.005	0.004	0.001	0.001	...	...

<sup>a</sup> Rubber.

<sup>b</sup> Tygon.

In another experiment, the instrument was operated according to standard procedure except that clean room air was pumped through the cylinder and then bubbled through the ampule. The first set was done with the piece of rubber tubing connecting the bubbler to the cylinder outlet tube that came with the machine. The second set was done with a piece of clean, new rubber tubing. The results are given in Table 6.

TABLE 6—*Effects of change in tubing on retention.*

Set	Trials	Dial Pointer Reading, %BA	Standard Deviation
Old tubing, rubber	10	0.004	±0.005
New tubing, Tygon	8	0.000	±0.007

H. *Condensation of Vapors in Trap and Tubing*—The instrument was operated according to standard procedure. Commercial saliva traps (mouthpieces) were attached to 22-cm lengths of rubber tubing. Vapors from a 0.40 percent ethanol solution which was held at 37 C in the breath simulator in a water bath were passed through the trap and tubing, which were at the specific temperature given in Table 7. The amount of condensate in the trap and tubing was determined by weighing. The results for six trials each are given in Table 7.

TABLE 7—*Effects of temperature of trap.*

Temperature of Trap, deg C	Dial Pointer Reading, %BA	Standard Deviation	Average Condensate, mg
20	0.332	±0.016	5.3
30	0.430	±0.007	1.8
45	0.474 <sup>a</sup>	±0.004	0

<sup>a</sup> This value, which seems too high for a 0.40 percent solution, is correct for 37 C. The Breathalyzer is calibrated to give a dial pointer reading of 0.4 %BA with the 0.40 percent ethanol at 35.5 C, which is the average temperature of breath as it leaves the mouth.

I. *Effect of Contaminated Room Air*—The instrument was operated according to standard procedure, but after the first reading a box was placed over the instrument. A paper towel dampened with 0.40 percent ethanol was placed under the box to allow ethanol vapor to diffuse into the open ampule. After 5 min another reading was made and the machine rezeroed. Then, air from near the ethanol dampened towel within the box was pumped through the sample cylinder and bubbled through the ampule. The results are given in Table 8.

TABLE 8—*Retention of ethanol contaminated room air.*

Trial	Dial Pointer Reading after Standing 5 min, %BA	Dial Pointer Reading after Pumping, %BA
1	0.013	0.003
2	0.016	0.008
3	0.013	0.004
4	0.012	0.001
5	0.003	0.002
6	0.004	0.004

J. *Effect of Stray Light*—The instrument was operated according to standard procedure using vapor from 0.40 percent ethanol as the sample was illuminated by a 250-W bulb in a 12-in. reflector 1.5 ft above the instrument. After the initial reading, an opaque hood was placed alternately over the reference ampule and the sample ampule. The results are shown in Table 9.

TABLE 9—*Effect of stray light on retention.*

Trial	Dial Pointer Reading, %BA		
	No Cover	Cover over Reference	Cover over Sample
1	0.108	0.108	0.108
2	0.111	0.111	0.111
3	0.139	0.139	0.139

K. *Dependence of the Instrument on the Line Voltage*—The instrument was operated in a standard manner but was attached to an autotransformer by which the line voltage to the instrument could be changed. The results of up to 20 measurements employing ethanol solutions at each voltage are shown in Table 10.

TABLE 10—*Effect of line voltage on retention.*

Voltage	Trials	Dial Pointer Reading, %BA	Standard Deviation
100	20	0.166	±0.003
105	17	0.164	±0.003
110	20	0.165	±0.006
115	20	0.168	±0.004
120	19	0.174	±0.004

In a separate trial, the instrument was operated at 115 V, and a sample of 0.40 percent ethanol was injected. The solution was allowed to come to a constant optical density (pointer reading of 0.185 %BA), and then the voltage was decreased to 30 V, at which point the light stopped glowing with no change in the reading. Upon increasing the voltage, the reading changed: from 0.185 %BA to 0.186 %BA at 133 V.

### Discussion

The first concern was whether the photoelectric system was stable and reliable. If the photovoltaic cells and electrical components could not give consistently reproducible readings, then the rest of the instrument's performance would be subject to those errors plus any other errors from other aspects of the instrument operation. Experiment A showed that consistent readings could be obtained. The 10- $\mu$ l sample had been used near the start of the study, and the standard deviation was larger than that for comparable experiments performed since then.

The percent BA scale was linear with respect to the mechanical and photoelectric systems over the entire range of the scale (experiment B). If the reaction follows Beers' law and the photometer has a linear absorbance output, then the scale should be linear. However, the readout scale could be calibrated to adjust for any deviation from linearity of the chemical or photometric systems. The photovoltaic cells in the Breathalyzer have an output which is linear with respect to the intensity of the illumination on the cells. The ratio of the intensity of illumination through an absorbing substance (the ampule of dichromate solution) compared to the intensity of illumination without the absorbing substance is the percent transmittance; thus, the response of the photovoltaic cells to changes in the transmittance of the solution is linear over the calibration range of the instrument. However, the balancing of the effect of light by that of distance from the photocell follows an inverse square law. The reciprocal of the square of the distance that the light is moved has a virtually linear relationship to the logarithm of the reciprocal of the change in transmittance, as measured by the photovoltaic cell over the small range of the instrument; thus, the light movement (and pointer readout) gives a linear measure of concentration. A similar effect is the basis for the Bunsen photometer.

The reliability of the chemical reaction in the instrument was tested by injection of dilute ethanol solution (experiment C). The standard deviations of the dial pointer readings were  $\pm 0.004$  or  $\pm 0.005$  %BA (Table 2), which is in the same range as the  $\pm 0.003$  to  $\pm 0.008$  found in experiment A. Thus, the reaction did not add any appreciable variation of uncertainty to the readings of the instrument.

The results in Fig. 1 further showed that the response of the combined chemical, photo, and mechanical systems is fairly linear with respect to added ethanol up to 0.250 %BA. There was a slight reduction in the reading at 0.308 %BA which, when corrected for the dilution caused by injection of the liquid sample, was actually 0.310 %BA.

The reaction of the dichromate with ethanol is reported to be complete within 1.5 min.<sup>4</sup> To verify this report, readings were taken near the 1.5-min time (experiment D) to find if the reaction was complete. The reaction was nearly complete after about 75 s, although a slow upward drift continued up to at least the 2-min mark (Fig. 2).

Since commercial ampules are made in large batches and stored for indefinite times in warehouses or in the police station prior to use, the stability of the chemicals in the ampules was checked (experiment E). Oven aging at 100 C was used to simulate long term aging at moderate temperatures. The data (Table 3) suggested a slight decrease in the reading for longer oven aging; however, the mean values are within the standard deviation ranges of the other values. Dichromate should not deteriorate in a sealed ampule at moderate temperatures and away from light. The ampules should be satisfactory for use after a storage time of many years if kept in the dark.

Because a different glass bubbler is used for each subject, there is a possibility of a variation in the orifice, which could cause a change in the rate of bubbling (experiment F). If a large orifice allowed the air from the cylinder to bubble through the ampule too fast, the absorption of alcohol might not be complete. Experiment F showed that the bubbling rate was independent of the orifice size until the orifice was smaller than normal (Table 4). Above that size, the rate of bubbling was controlled by a restriction in the line from the cylinder to the glass bubbler.

The absorption of ethanol vapor into the tubing could lead to error. The reading could be low on a sample put into a clean instrument, where ethanol would be removed from the sample by absorption. On the other hand, if the Breathalyzer is used immediately after a sample having a high concentration of ethanol has been tested, a sample with a weaker concentration might give a slightly high reading owing to previously absorbed ethanol being desorbed back into the air stream (experiment G).

In Table 5 the first column under pointer readings gives the value of ethanol that would be flushed from the instrument just prior to zeroing the instrument for the next determination. The second column gives the value of ethanol that would be carried into the sample being tested. The value is small but not negligible. Beyond the third flush of the system the values are negligible. However, in the case of the rubber tubing that was on the instrument when received, the residual values are consistently positive and just large enough to suggest a small carryover of ethanol. This suggests that the rubber tubing should be replaced periodically as part of a maintenance program for the instrument. Unless subjected to unusual conditions, the tubing should be good for at least six months between changes. The Tygon tubing did not appear to retain any more ethanol than the rubber tubing even though the Tygon is composed of more polar material than rubber and, therefore, should absorb more ethanol.

Table 6 gives the results of using clean room air as the sample. It shows that the slight positive values in the later flushes of the instrument in Table 5 are not significant.

Besides the absorption of ethanol by tubing in the instrument, there is the possibility of condensation of water and alcohol from a subject's breath (experiment H). Alveolar air is saturated with water vapor at nearly 37 C. When exhaled air passes through the mouth-piece and any tubing that is below the temperature at which air leaves the mouth (34 C), the air will be cooled and excess moisture will condense onto the cooler parts of the apparatus. Although the ethanol vapor is not anywhere near saturation, a part of it will

dissolve in the condensed water in accordance with the partition ratio between water and air at the temperature concerned. The data in Table 7 show that there was definitely a lower percent BA reading when some of the vapors were allowed to condense. This effect is not present during any routine checking of the instrument with a breath simulator, which uses 0.40 percent ethanol solution and maintains that part of the tubing which is not warmed by the instrument to within 2 C of ambient temperature. However, when a subject is being tested, his breath will, in most instances, be above room temperature and will thus enable condensation of the latter in the mouthpiece. The effect of this potential error is a reduced percent BA reading, which is not unfair to a subject being tested. Instrument operating instructions call for the storage of the intake tube inside the warmed portion of the instrument until just before sample introduction to minimize condensation in this location.

While in the instrument, the ampule is open to the atmosphere and presumably could absorb oxidizable vapors. These vapors might arise from breathing over the instrument, evaporation of spilled drinks from clothing, vapors of solvents from cleaning and polishing agents, paints and lacquers, or various aerosol sprays. The latter factors can be controlled by the operators of the instrument or by moving the portable instrument to another room. Ethanol vapor from the presence of a subject was studied in experiment I. The 5-min exposure under the box retaining ethanol vapor was longer than the time a subject would stand near the instrument while being tested. There was a definite uptake of ethanol during that time. Since the subject needs to be near the instrument less than 1 min to give his breath sample, and the uptake of vapors will be proportional to the time of exposure, a more realistic but not necessarily exact figure will be obtained by dividing the readings by five. Thus, a subject with a concentration of 0.40 %BA breathing over the Breathalyzer for 1 min could cause a positive reading on the order of 0.003 %BA (1/5 of trial 2, Table 8) which is within the standard deviation of the instrument.

The air that was contaminated with ethanol vapor and pumped through the instrument also gave a small but measurable response. This indicates that the preliminary flushing and zeroing of the instrument should be done with the subject at a distance. When clean laboratory air was flushed through the instrument (no chemicals had been used in the vicinity since the previous day), the response was negligible even with the old tubing (Table 6).

Ordinary room lights do not affect the readings as shown by the extraordinary light used in experiment J. Even when a lamp was held at the top of the instrument and directed at an angle toward the photocell, there was no deflection of the galvanometer.

The effect of variations in the line voltage on the performance of the instrument was of considerable interest. In any electrical supply there are possible fluctuations in voltage due to a heavy draw of current elsewhere in the building or especially from another outlet on the same circuit. The tests (experiment K) showed that the instrument gives essentially the same results when operated at voltages ranging from 100 to 120 V, since the single lamp affects both photocells by the same factor. It was further shown that voltage variations between the time that the instrument is zeroed and the time that the reading is made do not change the reading of the instrument even at 133 V, which is a surge far in excess of normal variations.

Another factor, which was not investigated experimentally because its effects can be readily calculated, involves temperature changes. The cylinder in which the sample is captured is held at 47 to 53 C according to standard procedure. If the temperature is high, the sample gases will expand, and thus there will be fewer molecules of air (and ethanol) retained in the essentially constant volume of the cylinder. The mathematical expression



of the law governing the behavior of gases is  $n = PV/RT$  where  $n$  = the number of gram molecular weights (moles) of the material in question,  $P$  = pressure in the system,  $V$  = the volume of the system,  $T$  = absolute temperature of the system, and  $R$  = the gas constant. In the Breathalyzer, the volume is constant so the equation may be expressed as  $n \propto P/T$ , or the amount of material varies directly with a change in pressure and inversely with a change in temperature. The operating range of the instrument is taken as  $50 \pm 3$  C ( $323 \pm 3$  K). Thus, the expected temperature variation would be 1 percent of the absolute value which carries over to an instrument reading at a defined point of intoxication, for example, at 0.100 this is  $\pm 0.001$  %BA, or an insignificant difference.

Variations in the ampule outside diameter could range from 1.588 to 1.650 cm (0.625 to 0.650 in.) according to the permissible variation in the ampule gage. Assuming a constant wall thickness, this represents a  $\pm 2$  percent variation in the absorbance which gives, as in the example above,  $0.100 \pm 0.002$  %BA, which is an insignificant difference.

Pressure variations caused by weather patterns or elevation make no difference since the subject and instrument are under the same pressure. The exhaled alveolar air is equilibrated, so the partial pressure of ethanol vapor in the alveoli will be a function of temperature only for any given blood alcohol concentration.

Without doing anything else to the instrument, the reading could be changed by as much as 0.012 %BA simply by moving the reference ampule in its socket. A simple device molded from an inert material such as polyethylene could be used in the socket to provide a friction fit of the ampule and prevent its moving in the socket. As long as the ampule is not moved after the instrument has been zeroed, the readings will not be affected. Another mechanical problem that was encountered was wobbling of the pointer in the metal knob. This was eliminated by rigidly securing the pointer in the knob with epoxy cement.

When the room temperature varied widely, readjustment of the thermostat was necessary to keep the instrument in the proper temperature range. Some corrosion was noted in the ampule socket but it had no adverse effect on the functioning of the instrument. Since sulfuric acid is corrosive to most metals, the routine maintenance of the instrument should include cleaning and lubricating any parts of the mechanism that might have had sulfuric acid spilled on them.

### Summary

The Breathalyzer is a satisfactory, sufficiently accurate, and reliable instrument for its purpose when maintained and used according to instructions. The commercial Breathalyzer reagent ampules which were tested were reliable. Slight variations in size or filling among those that pass the gage test are insignificant.

The method of transferring the breath sample to the Breathalyzer is adequate. There is some question of loss of sample by condensation of water and ethanol in the mouthpiece, but any error will be on the low side of the true blood alcohol value. To minimize condensation, the mouthpieces could be stored in the warmest place in a room or warmed prior to use.

A dial pointer reading consistently on the high side would be caused only by retention of ethanol vapor from a breath sample of high concentration to one of lower concentration run immediately afterwards. This effect can be minimized by giving the instrument a complete flush with room air immediately after finishing with a subject. Then, before taking a sample from the next subject, flush and zero the instrument once more, plus any additional number of times until the amount of change on the scale does not exceed a chosen value, such as 0.004 %BA.

Moving either the reference or the test ampule within their sockets after the instrument has been zeroed can cause a small positive or negative error. The use of a plastic insert to hold the ampules firmly would be a useful addition to the instrument.

The instrument should be checked regularly for corrosion resulting from spillage of the strongly acidic reagent contained in the test ampule, for looseness of the pointer, and for deterioration of the tubing.

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