Pyrex Suspensions in Turbidimetric and Colorimetric Determinations

Standards of Comparison for Bacterial Suspensions and in the Resazurin Test

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IT has been difficult in general laboratory practice to prepare reliable standards of comparison for turbidity and turbidity with color, because, while it is possible to establish an adequate color-range with inorganic compounds in acid solution, materials for the simulation of turbidity are neither so readily available nor so satisfactory. In 1939 Brewer and Cook[1] reported that a suspension of Pyrex glass had been found to be markedly superior in the standardization of typhoid vaccine to the suspensions of silica or barium sulfate ordinarily employed as standards of turbidity. Particles of from 0.5 to 3.5 microns in diameter were suspended in distilled water to simulate suspensions of typhoid bacilli; they did not dissolve or aggregate during a 5 year period of storage. The technic of Brewer and Cook has been adapted to the preparation of stable permanent standards of turbidity in the preparation of bacterial suspensions.[2] Pyrex suspensions in colored media have also been used experimentally as color standards in the resazurin test used in the grading of milk.[3] Other possible uses of the suspensions are as standards of comparison for milk of varying natural colors; alpha-naphthoflavone-iodine adsorption compound, a colored suspension that is formed in a test for residual chlorine in water[4]; colloidal complexes of copper or zinc with sodium diethyldithiocarbamate; mixed suspension of silver chloride and silver chromate found at the endpoint of the titration of chloride in water; precipitate of barium sulfate encountered with turbidimetric tests for sulfate or barium. For this latter purpose it would be necessary to modify the procedure of decantation to select the larger particles.

BACTERIAL SUSPENSIONS

The modified technic differs from the original in that it employs a longer period of shaking, by which a suspension may be made to the required density without the tedious process of evaporation, and that standardization is by photoelectric colorimeter instead of by particle counting under the microscope. Variations in particle size are limited by specifying both the turbidity and concentration of the stock suspension. In the experimental studies, series of standards were made for direct visual comparison with various bacterial suspensions — pneumococcus, meningococcus, *Bacterium typhosum*. Turbidity was made equivalent to that of the barium sulfate standards then used[5] and the suspensions were arbitrarily numbered in ascending order as concentration was increased. Each Pyrex standard was prepared in distilled water and in two colored solutions to match the media used for the bacterial suspensions. Without exception, com-

parisons were made more readily with Pyrex suspensions because of their similarity to the sample in color, particle size, and turbidity. Pyrex standards have now replaced barium sulfate standards. The rate of settling is so slow that frequent shaking is unnecessary. Stability during storage is an added advantage.

PREPARATION OF STOCK SUSPENSION OF PYREX GLASS

Break discarded Pyrex glassware into ¾" or smaller chips. Fill a Pyrex bottle, 6¾" in diameter and fitted with a Pyrex glass stopper, to a depth of 2½" with chips. Clean the chips and bottle. Just cover the chips with distilled water and shake for two 6 hour periods in a machine operating at 130 cycles per minute with a 2" stroke which should provide for violent agitation of the chips. Use fresh water for each period of shaking. Again cover the chips with distilled water and shake for the third time until the suspension approximates the opaqueness of skim milk; the time required will vary from 24 to 120 hours (not necessarily consecutive), depending upon whether freshly broken chips are used.

Transfer the suspension to 1 liter cylinders, cover, and let stand for 48 hours to allow the coarser particles (2 microns) to settle. Decant supernate; allow to settle 24 hours and decant again. Determine the turbidity of the decanted suspension in a photoelectric colorimeter, Luximeter (L) or Klett-Summerson (KS). Use test tubes and a blue filter with the latter. Dilute with distilled water if necessary to bring L to 40 or KS to 590. If too dilute, return the suspension to the original bottle containing the glass chips, shake further in the machine, repeat the settling and decantation procedure, dilute to the standard turbidity, and determine the dry-solids content. If less than 0.20 per cent, return the suspension to the cylinders used for the second decantation, mix, and let settle for a shorter period to retain a higher proportion of large particles; if more than 0.25 per cent, use a longer settling period to retain a lower proportion. (The suspension we have actually used is 0.22 per cent. How closely this should be approximated would depend upon the precision required.)

Standards for Bacterial Suspensions

Prepare standards by dilution and calibration in a photoelectric colorimeter as indicated in Table 1. The diluent is distilled water or a colored medium.

Make standards resembling beef-extract or bile-peptone solutions by mixing one volume of diluted stock Pyrex suspension with two volumes of the color solution. Use a dilution of one volume of distilled water with two volumes of color solution for the zero setting of the colorimeter that is used. These suspensions settle slowly and should be mixed after several hours' standing.

Beef-extract broth color solution—Dissolve 1.245 gm. of potassium chloroplatinate (K_2PtCl_6) and 1 gm. of cobalt chloride ($CoCl_2.6H_2O$) in distilled water. Add 100 ml. of concentrated hydrochloric acid and dilute to 1 liter.

Peptone-bile color solution—Dissolve 2 gm. of potassium chloroplatinate (K_2PtCl_6) and 3.75 gm. of copper sulfate ($CuSo_4.5H_2O$) in distilled water. Add 100 ml. of concentrated hydrochloric acid and dilute to 1 liter.

Dispense the suspensions in round-bottomed vials, 15 or 19 mm. outside diameter, marked

TABLE 1

Dilution and Calibration of Pyrex Suspensions

Turbidity Standard	*Approximate Concentration of Stock Suspension*	*Turbidity as Indicated by Photoelectric Colorimeter Scale Readings*	
		Luximeter	*Klett-Summerson*
No.	Per cent	L	KS
0.5	3.32	95	40
1	8.34	89	100
2	18.8	78	210
3	30.0	70	290
4	39.6	63	360
5	52.2	57	420
6	60.5	53	460
7	66.7	50	490

at the initial liquid level (bottom of meniscus), and equipped with moulded screw caps. Since these colored standards are acid, the cap lining must be acid resistant. Examine the suspensions with photoelectric colorimeter at intervals of 6 months, or whenever the level of the liquid becomes lower than the graduation mark on the vial, and adjust the concentration if necessary.

RESAZURIN TEST OF MILK

Successful application of the resazurin test for the grading of raw milk according to sanitary quality is dependent upon reliable standards for color comparison in the range from blue through shades of purple to red, to pink, and to white. It has been found difficult in this laboratory to prepare resazurin-treated milk representing the maximum blue or red upon which Ramsdell based the preparation of his standards.[3] Experience with the Pyrex turbidity standards encouraged the hope that these suspensions might also be used as permanent standards simulating milk. The stock suspension was allowed to settle only long enough to remove the coarsest particles. Inorganic color solutions were added to provide a series with which any of the colors encountered in the test could be matched. These standards, given arbitrary numerical designations, were used satisfactorily in a preliminary study of the resazurin test and the resazurin-rennet test[6] in natural daylight or under artificial reflected light—a fluorescent "daylight" lamp. They were also used in the field for a year and showed no deterioration in careful comparison with freshly prepared standards.

No attempt has been made to correlate with milk-quality the arbitrary numbers assigned to the color standards. Rather, it is hoped that this correlation and also standardization in terms of an accepted color-recording system may be undertaken by investigators who are studying the test. Certainly the utility of the resazurin test will be more readily determined if the standards can be easily prepared in any analytical laboratory. Davis[7] has suggested that the establishment of a color standard be delegated to an international committee.

White Suspension—With distilled water prepare a suspension concentrated to resemble skim milk as described in the preparation of Pyrex standards for bacterial suspensions; allow to settle only 5 minutes to remove the coarsest particles. Adjust the concentration by dilution or further shaking so that 1 ml. in 100 ml. has a Luximeter reading of 83 or 84 or a Klett-Summerson reading of 160 to 150.

Blue Suspension—Prepare a concentrated white Pyrex suspension. Adjust so that 1 ml. in 100 ml. has a Luximeter reading of from

TABLE 2

Preparation of Permanent Standards for the Resazurin Test

Color Range	*Turbidity Standard*	*White Suspension*	*Blue Suspension*	*Red Solution*	*Yellow Solution*
	No.	ml.	ml.	ml.	ml.
	13	1.85	3.00	0.15	0.00
Blue	15	2.84	2.00	0.16	0.00
to	17	3.83	1.00	0.17	0.00
red	19	4.82	0.00	0.18	0.00
to	21	4.87	0.00	0.13	0.00
milk	23	4.93	0.00	0.07	0.00
color	25	4.98	0.00	0.00	0.025

Note: In the Munsell Color Notation[8] the turbidity standards were interpreted as follows: No. 13, [5.0 PB] 7/3; No. 15, [7.5 PB] 7.5/4; No. 17, [P] 8/2; No. 19, [2.5 RP] 8/4; No. 21, [5 RP] 8/4. The delicate gradations from No. 17 could not be indicated with the same exactness as for the lower numbers. It would seem that advantage could be taken of the highly developed Munsell notation if a practical field outfit could be developed, for example, colored strips mounted in test tubes for comparable examination with the treated sample.

80 to 82 or a Klett-Summerson reading of 190 to 170. Dissolve 15 gm. of copper sulfate ($CuSo_4.5H_2O$) in about 75 ml. of the suspension, add 1.6 ml. of concentrated sulfuric acid, and dilute to 100 ml. with more of this suspension. This should have a Luximeter reading in the same range as the white suspension in a 1:100 dilution. The Luximeter should be adjusted to 100 with a distilled water solution of 15 gm. of copper sulfate and 1.6 ml. of sulfuric acid in 100 ml. Adjust the turbidity, if necessary, by preparing suspensions of higher or lower Pyrex content, maintaining the same concentrations of copper sulfate and acid.

Red Solution—Prepare a distilled water solution of 476 gm. of cobalt chloride ($CoCl_2.6H_2O$) and 100 ml. of concentrated hydrochloric acid per liter of solution.

Yellow Solution—Prepare a distilled water solution of 2 gm. of potassium dichromate ($K_2Cr_2O_7$), 38 gm. of cobalt chloride ($CoCl_2.6H_2O$), and 8 ml. of concentrated hydrochloric acid per 100 ml. of solution.

Dispense the standards in vials of 15 mm. outside diameter having screw caps with acid-resistant linings and a graduation mark at the initial liquid level (top of meniscus). Whenever the liquid level is low due to evaporation, make up to volume with distilled water. At intervals of 6 months or at any time when portions of the suspension have become so packed that they cannot be resuspended, discard and replace with freshly prepared standards.

SUMMARY

Methods are given for the preparation of Pyrex glass suspensions as standards of turbidity.

Pyrex suspensions in colored media have been substituted for suspensions of barium sulfate in the standardization of bacterial suspensions and have been employed experimentally in the resazurin and resazurin-rennet tests for the grading of milk. The glass suspensions closely resemble bacterial suspensions in appearance, settle slowly, and remain stable longer than those of barium sulfate. In the resazurin test, Pyrex suspensions in colored media have the advantage of being directly reproducible from readily available materials. Standardization in terms of an accepted color-recording system and in relation to milk-quality is suggested.

Pyrex standards have been found to be stable for at least one year.

Various uses for Pyrex suspensions are suggested.

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