

ACCELERATED RESAZURIN REDUCTION TEST FOR MILK

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AMONG the platform tests used for the rapid detection of poor quality milk supplies, received at the dairy plant or collection centre, the ten minutes resazurin test has been considered to be the most satisfactory method available for picking out samples having excessive numbers of bacteria and leucocyte cells.^{1,2} The need for adopting a much quicker method of testing milk supplies on the receiving platform is, however, widely recognised. In the course of the studies on the development of a suitable bacteriological test for the above purpose, the possibility of shortening the time taken for reduction of resazurin or methylene blue in milk by stimulating or accelerating the dehydrogenase activities of bacteria has been explored.

A number of substances, e.g., B. complex vitamins, amino acids, sugars, hydrolysates of casein, commercial peptones, yeast extract and beef extract, which are known to stimulate bacterial growth and metabolism, were added to milk and the effect on the reduction of resazurin and methylene blue was studied. Among the materials tried out, riboflavin was found to cause a slight acceleration in the reduction of the indicators when milk contained a preponderance of particular species of bacteria. Reducing agents such as ascorbic acid, cysteine and glutathione caused a marked reduction of the indicators but they were effective even in

pasteurised and boiled milks in the absence of any bacterial activity. It was, however, interesting to find that the addition of certain commercial peptones, yeast extract and beef extract in small concentrations had a very marked effect on the reduction of O/R indicators, particularly resazurin, in milk containing large numbers of actively growing bacteria. The addition of these materials to raw milk, containing very few bacterial cells, also showed some accelerating effect on the reduction of resazurin presumably due to the activation of some milk enzymes, but this effect was not produced in pasteurised and boiled milks. Paper chromatographic analysis of the peptone or yeast extract revealed that one particular component, which moved to a position corresponding to R_f value of about 0.51 in the chromatogram (butanol-acetate-water) appeared to be the activating factor.

The results of representative trials showing the effect of addition of peptone in different concentrations to milk samples, containing different levels of bacterial population, on the reduction of methylene blue and resazurin are shown in Tables I and II. It may be seen that the reduction of both the indicators is markedly accelerated by the addition of peptone and that this effect is progressively intensified with increase in the concentration of peptone as well as in the numbers of bacteria.

TABLE I

Effect of adding Stearn's peptone on the reduction of methylene blue and resazurin in milk*

Milk sample	Standard plate count per ml.	Methylene blue reduction time		Resazurin reduction disc numbers (Lovibond)					
				Without peptone			With peptone		
		Without peptone	With peptone	At the end of			At the end of		
				2 min.	10 min.	60 min.	2 min.	10 min.	60 min.
	(Millions)	(Hr. Min.)	(Hr. Min.)	(Disc Numbers)					
1	0.017	5-10	5-0	6	6	5½	6	5½	5
2	0.254	4-50	4-40	6	5½	5½	6	5½	5
3	0.450	3-50	3-15	6	5½	4½	6	5½	4
4	3.250	2-10	1-15	5½	5	3½	5½	5	3
5	20.500	0-22	0-15	5½	4	3	3	1	0
6	45.000	0-13	0-2	5	2½	0	½	0	0
7	91.000	0-8	0-2	4	½	0	0	0	0
8	150.000	0-5	0-1	4	½	0	0	0	0

* *Peptonum Siccum Stearns* (Frederick Stearns & Co., Detroit, U.S.A.).

Concentration: 0.2% (0.2 ml. of 10% peptone solution in 10 ml. milk).

TABLE II

Effect of adding Stearns peptone in different concentrations on the reduction of resazurin in milk

Milk sample	Standard plate count per ml.	Incubation time	Concentration of peptone				
			0.0	0.05	0.10	0.20	0.25
	(Millions)	(Minutes)	(Resazurin disc numbers)				
1	0.019	2	6	6	6	5½	5½
		10	5½	5½	5½	5	5
2	0.225	2	6	6	5½	5	4½
		10	5½	5½	5	4½	3½
3	6.110	2	5½	5	4½	4	2
		10	4½	4	2½	2½	1
4	33.000	2	5½	3½	2	½	½
		10	4	1½	1	0	0

It was also found that the resazurin disc numbers of milk samples containing peptone at the end of two minutes were generally comparable with the readings given by the corresponding control samples on the ten minutes reduction test. Thus, by adding a suitable concentration of the peptone, it may be possible to shorten the incubation time for the resazurin reduction test to less than five minutes and obtain information regarding the quality of milk supplies on the basis of which they may be accepted or rejected on the receiving platform. Based on these observations, a two minutes accelerated resazurin reduction test for milk has been developed and the results of some trials carried out to grade milk samples by the

new test as compared to the ten minutes resazurin test are shown in Table III.

TABLE III

Comparative grading of milk supplies by the Ten minutes and Two minutes (Accelerated) Resazurin tests

Grading by ten minutes Resazurin test (Disc number)	Number of samples	Distribution of samples according to Resazurin disc numbers in the two minutes (accelerated) test (Disc numbers)				
		6, 5 & 4	3½ to 2	1½ to 1	½ & 0	
1 6, 5 & 4 (Good)	21	14	5	1	1	
2 3½ to 2 (Fair)	18	1	7	6	4	
3 1½ to 1 (Poor)	6	6	
4 ½ & 0 (Very poor)	17	17	

Further investigations are in progress to isolate the particular fraction of peptone responsible for accelerating the reduction of resazurin in milk and to study the practical application of the method for the rapid bacteriological examination of milk supplies on the receiving platform.

1. Ministry of Agriculture and Fisheries, England, (1942), Form C. 150 (T.P.Y.), H.M.S.O., London.
2. Gurbhagwant Singh, Laxman Rao, M. R. and Laxminarayana, H., *Indian J. Dairy Sci.*, 1948, 1, 11.

SOME IGY DATA ON ANTARCTICA

THE scientific exploration projects of the thick, resting on bed rock 8,200 ft. below sea-level Antarctic and the south polar region under level. This may be the thickest ice measured the IGY programme have led to an accumulation anywhere in the world. Deep drilling operations of data on such wide ranges of subjects as aurora to more than 1,000 ft. depth have brought up and airglow, ionospheric physics, cosmic rays, cores of ice which fell as snow over 1,000 years geomagnetism, oceanography, glaciology, gravity ago. Analysis of the ice cores is expected to measurement and special studies in botany, throw more light on past climates. zoology and microbiology. These data from A record low temperature of -125.3° F. was groups of observers from different stations, when reported by the USSR-IGY station near the analysed, co-ordinated and published are bound centre of the continent. It was reported that to add greatly to our knowledge of the ice-during the Antarctic mid-summer the south pole sheathed continent. receives more sunlight than any other place on

Seismic measurements of ice thickness indicate earth. But as nearly 95% of the radiation is that there is probably 40% more ice in the reflected by the ice cover the pole itself is left Antarctic than previously thought. The U.S.- one of the coldest spots in the world.—*Bull. Am. IGY team at Bryd Station measured ice 14,000 ft. Met. Soc.*, January 1959.