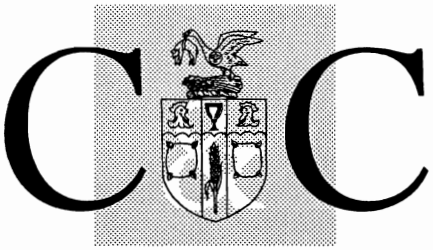
R&D REPORT NO. 32

Evaluation of 3M Petrifilm Count Plates for Enumeration of Enterobacteriaceae and Coliforms from Food Samples

November 1996



Campden & Chorleywood
Food Research Association

Campden & Chorleywood
Food Research Association

Director-General
Prof. C. Dennis
BSc, PhD, FIFST

Chipping Campden
Gloucestershire
GL55 6LD UK
Tel: +44 (0)1386 842000
Fax: +44 (0)1386 842100

R&D Report No. 32
Project No. 25141

Evaluation of 3M Petrifilm Count Plates for Enumeration of Enterobacteriaceae and Coliforms from Food Samples

S. MacPheee, A.R. Bennett and R.P. Betts

November 1996

CONTENTS

SUMMARY

Petrifilm count plates can offer routine microbiology laboratories a simple, labour-saving alternative to the conventional pour-plate technique. The plates are ready to use, quick and easy to inoculate, and require minimum storage and incubator space. There is no necessity for preparation of media, and since there is no requirement for holding molten agar at a controlled temperature, no risk of heat shock to injured organisms. Petrifilm plates are available for a range of organisms, including coliforms and Enterobacteriaceae.

The Petrifilm Coliform Count (CC) plate and the Petrifilm Enterobacteriaceae Count (EB) plate were compared against conventional methods (BS/ISO) for enumeration of coliforms and Enterobacteriaceae in foods. 117 food samples were tested for naturally occurring and inoculated coliforms, and 33 samples were simultaneously tested for naturally occurring Enterobacteriaceae. Counts of presumptive coliforms on the Petrifilm CC plate, and presumptive Enterobacteriaceae on the Petrifilm EB plate, gave equivalent data with high correlation coefficients when compared with standard BS/ISO methods using pour plates of VRBA (BS 5763, ISO 4832) and VRBGA (BS 5763, ISO 7402) although at low levels the Petrifilm EB plate tended to over estimate numbers compared with VRBGA. Correlation coefficients for the data for coliforms and Enterobacteriaceae were 0.98 and 0.94 respectively.

INTRODUCTION

Routine microbiological analysis of foods often includes the enumeration of coliforms and/or Enterobacteriaceae. Conventional methodology involves inoculating a sample of food homogenate into Violet Red Bile Agar (VRBA) for coliforms and Violet Red Bile Glucose Agar (VRBGA) for Enterobacteriaceae. A pour plate with overlayer method is used in both cases, and following incubation at 37°C for 24h, characteristic colonies are counted to give presumptive counts of the organisms (BS 5763, Part 2:1991, ISO 4832 and BS5763, Part 10, ISO 7402).

The Petrifilm Coliform Count (CC) plate and the Petrifilm Enterobacteriaceae Count (EB) plate have been developed by the 3M Corporation as ready-to-use alternatives to conventional methodology. Petrifilm CC plates contain violet red bile nutrients, a cold water soluble gelling agent, and a tetrazolium indicator dye which facilitates colony enumeration. An aliquot of diluted food sample is inoculated onto the gel surface of the plate. The top film, which is coated with the indicator dye and gelling agent, is placed over the inoculum and the inoculum is spread evenly over the surface by gently pressing the top of the plate with a plastic spreader provided with the plates. The gel solidifies in one minute and plates are then incubated at 37°C for 24h. Coliforms ferment lactose in the medium to produce gas, which is trapped around the colony and thus allows differentiation of coliforms from other Gram-negative bacteria.

In Petrifilm EB plates, in addition to gas, fermentation is demonstrated by acid production, shown by a colour change in the indicator due to a decrease in pH, resulting in a yellow zone surrounding the colony. Enterobacteriaceae may produce gas or acid or both gas and acid, and are thus differentiated from non-fermenting non-Enterobacteriaceae colonies.

This document reports data on the evaluation of Petrifilm CC and Petrifilm EB plates. Food samples were analysed at CCFRA for naturally occurring and artificially inoculated coliforms and Enterobacteriaceae by the Petrifilm methods and by the conventional British Standard (ISO) methods.

MATERIALS AND METHODS

Food Samples

117 food samples were purchased from a variety of local retail outlets in order to obtain a wide variation in contamination of the products. The food samples comprised raw beef (17), raw minced pork (1), raw poultry (12), raw sausages (6), cheese (14), cream (1), desserts and cakes (6), yogurt (3), chocolate (2), pre-packed vegetables (29), ready meals (6), salads with dressing (3), pâté (2), pre-cooked prawns (2), tuna (3), cooked ham (4), and cooked meat products (6).

A selection of these foods were subjected to temperature abuse by holding overnight at ambient temperature (approximately 20°C) in order to increase the levels of natural flora.

A further selection of foods were inoculated with freeze-stressed *E. coli*. Inocula were prepared by culturing *E. coli* Campden & Chorleywood Food Research Association (CCFRA) strains 3482, 4104, 3900, 3901 and 3496 in Nutrient Broth (NB) at 37°C for 24h. Broths were frozen at -40°C, then thawed to cause freeze injury to the cells. Food samples (20g) were inoculated with between 5×10^0 and 5×10^4 *E. coli* cfu/g of sample.

Evaluation Procedure

Each food was analysed for the enumeration of coliforms using a conventional method (BS5763: Part 2) and Petrifilm Coliform Count (CC) plate method. In addition, 33 foods were analysed for the enumeration of Enterobacteriaceae using a conventional method (BS 5763: Part 10) and Petrifilm Enterobacteriaceae Count (EB) plates.

Samples (20g) of foods were homogenised in 180ml Maximum Recovery Diluent (MRD) in a stomacher for 1 minute. Ten-fold dilutions were made in MRD and duplicate aliquots (1ml) of appropriate dilutions were used for each enumeration method.

Conventional Method for Enumeration of Enterobacteriaceae: BS5763:10, ISO 7402

Aliquots (1ml) of diluted food samples were plated onto Violet Red Bile Glucose Agar (VRBGA, LabM, LAB88) using a pour plate with overlayer method. Plates were incubated at 37°C for 24h. After incubation, purple/red colonies greater than 0.5mm in diameter, with or without haloes, were counted and calculated as presumptive Enterobacteriaceae cfu/g of sample.

Conventional Method for Enumeration of Presumptive and Confirmed Coliforms: BS5763:2, ISO 4832

Aliquots (1ml) of diluted food samples were plated onto Violet Red Bile Agar (VRBA, Merck 1406) using a pour plate with overlayer method. Plates were incubated at 37°C for 24h. After incubation, purple/red colonies with or without a purple/red halo greater than 0.5mm in diameter were counted and calculated as presumptive coliforms cfu/g of sample.

For 20 food samples, presumptive colonies were confirmed by inoculating five typical colonies into MacConkey Broth containing a durham tube. Broths were incubated at 37°C for 24h, then examined for acid and gas production. Acid and gas-producing isolates were considered to be coliforms. From the number of confirmed colonies the count of confirmed coliform cfu/g was calculated.

Petrifilm Method for Enumeration of Presumptive Enterobacteriaceae

Aliquots (1ml) of diluted food samples were inoculated onto the guar surface of Petrifilm Enterobacteriaceae Count (EB) plates. The top film of the plate was rolled down onto the sample, avoiding the entrapment of air bubbles. The inoculum was distributed evenly by gently pressing the plastic spreader (provided with the Petrifilm media) onto the centre of the top film. Plates were left undisturbed for at least 1 minute to allow the gel to solidify. Plates were incubated at 37°C for 24h.

After incubation, plates containing <100 colonies were counted. Colonies were counted as follows.

(a) Red colonies with yellow zones of acid production.
(b) Red colonies with associated gas bubble.
(c) Red colonies with associated gas bubble and yellow zone.

The number of presumptive Enterobacteriaceae was calculated from the sum of the counts of colonies associated with acid and/or gas (a + b + c).

Petrifilm Method for Enumeration of Presumptive and Confirmed Coliforms

Aliquots (1ml) of diluted food samples were plated onto the guar film of Petrifilm Coliform Count (CC) plates. The top film was rolled down onto the inoculum, avoiding the entrapment of air bubbles, and the inoculum was spread evenly by pressing the plastic spreader gently onto the top film. Plates were left for at least 1 minute to allow the gel to solidify. Plates were incubated at 37°C for 24h.

After incubation, plates containing <150 colonies were counted. All red colonies, with or without gas, were counted and calculated as presumptive coliforms. A separate count was made of red colonies with an associated gas bubble and calculated as confirmed coliform cfu/g.

Analysis of Data

Results were analysed by linear regression, using data where real counts were obtained for both methods, comparing counts obtained on Petrifilm with counts obtained using the standard methods.

RESULTS AND DISCUSSION

Coliforms

Results of counts of coliforms on Petrifilm CC plates and VRBA are shown in Tables 1 and 2 and are expressed graphically in Figures 1 and 2. Figure 1 shows the comparison between counts of presumptive coliforms on Petrifilm CC plates and VRBA. Figure 2 compares counts of confirmed coliforms on Petrifilm CC plates with counts of presumptive coliforms on VRBA.

Counts of presumptive coliforms on Petrifilm CC plates and VRBA were generally similar (Table 1). The correlation coefficient for the data is 0.98 and the correlation line lies very close to the line of equivalence (Figure 1), indicating an excellent correlation between the data. There are a few discrepancies, however, at lower contamination levels, where Petrifilm tends to over count.

When comparing counts of confirmed coliforms on Petrifilm CC with counts of presumptive coliforms on VRBA, the correlation is good (correlation coefficient = 0.91), but the correlation line lies below the line of equivalence, indicating that counts on Petrifilm tend to be lower than counts on VRBA (Figure 2). This is an expected observation when confirmed counts are compared with presumptive counts. The comparison is shown to highlight that, although the difference is not great, different data is expected when using the Petrifilm CC for confirmed counts compared to those obtained with standard procedures. A laboratory using such a system must be aware of the reduced counts that would be obtained as the nature of the test is altered.

A comparison between confirmed counts on Petrifilm CC and confirmed counts on VRBA is shown in Figure 3. There is a good correlation between counts (correlation coefficient = 0.90) although counts on Petrifilm again tended to be slightly lower than counts on VRBA. More data is required if a definitive comparison of confirmed counts is to be made.

Enterobacteriaceae

Results of counts of Enterobacteriaceae on Petrifilm EB plates and VRBGA are shown in Table 3 and Figure 4. Figure 4 shows the comparison between counts of presumptive Enterobacteriaceae on Petrifilm EB plates and VRBGA. There is a good correlation between counts (correlation coefficient = 0.94). At lower contamination levels, counts on Petrifilm tended to be higher than counts on VRBGA, but at higher contamination levels ($>10^4$ cfu/g) the counts on both agars tended to be more evenly distributed around the line of equivalence.

TABLE 1

Comparison Between Counts of Coliforms on Petrifilm and VRBA

Food	Coliforms cfu/g		
	VRBA	Petrifilm CC	
	Presumptive	Presumptive	Confirmed
Minced Beef	7.9×10^5	6.2×10^5	4.9×10^5
Minced Beef	8.2×10^6	$6.5. \times 10^6$	2.0×10^3
Minced Beef	5.9×10^4	4.0×10^4	4.5×10^2
Minced Beef	2.5×10^2	1.0×10^2	5.0×10^1
Minced Beef	4.5×10^5	5.8×10^5	1.5×10^4
Minced Beef	1.2×10^6	1.2×10^6	4.5×10^4
Minced Beef	1.7×10^6	2.6×10^6	5.0×10^4
Minced Beef	1.5×10^6	2.2×10^6	2.5×10^4
Minced Beef	2.3×10^6	3.9×10^6	1.5×10^5
Minced Beef	1.9×10^6	2.3×10^6	6.5×10^4
Minced Beef	5.0×10^7	3.9×10^7	2.5×10^6
Minced Beef	3.0×10^6	3.3×10^6	$<5.0 \times 10^4$
Beef	9.6×10^4	6.0×10^4	$<5.0 \times 10^2$
Beef	1.4×10^5	7.0×10^4	$<5.0 \times 10^3$
Beef	1.9×10^6	7.3×10^5	$<5.0 \times 10^3$
Beef	4.2×10^6	2.1×10^6	$<5.0 \times 10^4$
Beef	9.0×10^6	3.0×10^6	$<5.0 \times 10^5$
Minced Pork	5.0×10^2	5.0×10^2	$<5.0 \times 10^2$
Diced Turkey	1.0×10^4	1.9×10^4	1.7×10^3
Diced Turkey	1.2×10^5	1.4×10^5	$1.1. \times 10^4$
Chicken	2.6×10^4	3.2×10^4	2.5×10^3
Chicken	1.5×10^2	8.2×10^3	3.0×10^1
Chicken	1.1×10^5	5.0×10^4	$<5.0 \times 10^3$
Chicken	4.8×10^3	6.7×10^3	5.0×10^2
Chicken	2.1×10^3	3.1×10^3	7.8×10^2
Chicken	3.0×10^3	3.7×10^3	5.8×10^2
Chicken	3.9×10^3	3.3×10^3	$<5.0 \times 10^1$
Chicken	4.1×10^4	4.3×10^4	$<5.0 \times 10^2$
Chicken Burger	5.2×10^2	1.2×10^3	8.5×10^1
Chicken Burger	6.7×10^3	5.0×10^3	2.8×10^2
Sausages	5.0×10^0	4.5×10^1	1.5×10^1
Sausages	3.5×10^1	$1.1. \times 10^2$	9.0×10^1
Sausages	1.3×10^3	6.4×10^3	5.0×10^1
Sausages	$<5.0 \times 10^0$	1.0×10^1	$<5.0 \times 10^0$
Sausages	9.1×10^2	3.6×10^2	2.0×10^2
Sausages	2.9×10^0	2.6×10^0	2.3×10^0

VRBA = Violet Red Bile Agar

Petrifilm CC = Petrifilm Coliform Count Plate

Presumptive count on Petrifilm CC = count of all red colonies

Confirmed count on Petrifilm CC = count of red colonies with associated gas bubble.

TABLE 1 CONTINUED

Comparison Between Counts of Coliforms on Petrifilm and VRBA

Food	Coliforms cfu/g		
	VRBA	Petrifilm CC	
	Presumptive	Presumptive	Confirmed
Brie	2.0×10^1	3.0×10^1	3.0×10^1
Garlic Brie	4.5×10^2	5.1×10^2	1.7×10^2
Cottage Cheese & Chives	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Parmesan	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Goats' Cheese	2.6×10^3	$4.4. \times 10^3$	$2.3. \times 10^3$
Camembert	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cheese	7.5×10^2	7.9×10^2	1.4×10^0
Cheese	4.0×10^2	3.0×10^2	3.0×10^2
Cheese	2.2×10^7	1.3×10^7	$<5.0 \times 10^4$
Cheese	6.3×10^3	6.8×10^3	$<5.0 \times 10^1$
Cheese	1.1×10^4	8.1×10^3	$<.5.0 \times 10^1$
Cheese	2.4×10^5	4.7×10^5	2.5×10^4
Cheese	2.4×10^7	$4.1. \times 10^7$	2.5×10^6
Cheese	2.5×10^7	2.7×10^7	$2.5. \times 10^5$
Cream	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cream Cake	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cream Cake	9.2×10^2	1.6×10^2	$<5.0 \times 10^0$
Cream Cake	2.5×10^3	5.5×10^3	7.0×10^2
Cream Tart	1.5×10^5	1.8×10^5	1.8×10^5
Trifle	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Trifle	8.0×10^1	5.7×10^2	2.5×10^1
Yoghurt	1.3×10^2	1.1×10^2	$<5.0 \times 10^0$
Yoghurt	9.0×10^1	8.0×10^1	$<5.0 \times 10^0$
Yoghurt	2.0×10^2	2.0×10^2	$<5.0 \times 10^0$
Chocolate	1.6×10^2	2.3×10^2	$<5.0 \times 10^1$
Chocolate	4.0×10^2	4.0×10^2	$<5.0 \times 10^1$
Stir Fry Veg	$8.5. \times 10^7$	6.7×10^7	$2.1. \times 10^5$
Stir Fry Veg	$4.3. \times 10^6$	5.8×10^6	6.1×10^5
Stir Fry Veg	7.8×10^6	7.8×10^6	1.8×10^6
Stir Fry Veg	3.5×10^6	$6.6. \times 10^6$	1.4×10^6
Beansprouts	7.1×10^6	1.4×10^7	$4.3. \times 10^6$
Beansprouts	2.8×10^7	4.4×10^7	1.4×10^7
Beansprouts	9.8×10^6	1.2×10^7	6.5×10^5
Beansprouts	1.0×10^7	1.4×10^7	4.0×10^6
Beansprouts	1.5×10^7	1.6×10^7	3.3×10^6

VRBA = Violet Red Bile Agar

Petrifilm CC = Petrifilm Coliform Count Plate

Presumptive count on Petrifilm CC = count of all red colonies

Confirmed count on Petrifilm CC = count of red colonies with associated gas bubble.

TABLE 1 CONTINUED

Comparison Between Counts of Coliforms on Petrifilm and VRBA

Food	Coliforms cfu/g		
	VRBA	Petrifilm CC	
	Presumptive	Presumptive	Confirmed
Beansprouts	1.9×10^7	4.5×10^7	9.5×10^6
Beansprouts	3.0×10^7	3.9×10^7	5.6×10^6
Beansprouts	3.5×10^7	6.4×10^7	8.5×10^6
Beansprouts	5.9×10^7	4.9×10^7	5.0×10^6
Beansprouts	7.6×10^2	4.3×10^2	1.0×10^1
Watercress	2.0×10^3	1.1×10^4	2.5×10^3
Watercress	2.4×10^3	4.9×10^3	2.4×10^2
Watercress	8.2×10^4	1.2×10^5	4.5×10^4
Watercress	8.5×10^5	1.8×10^6	5.4×10^5
Watercress	2.7×10^4	6.0×10^4	1.0×10^3
Lettuce	1.4×10^5	3.3×10^5	3.0×10^1
Lettuce	1.0×10^2	1.9×10^4	$<1.0 \times 10^2$
Mixed veg	4.9×10^5	1.9×10^6	$<5.0 \times 10^3$
Carrot and Cheese	5.3×10^3	9.5×10^3	1.9×10^3
Coleslaw	3.7×10^3	8.5×10^3	$<5.0 \times 10^0$
Bean/Pasta Salad	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Apple/Nut Salad	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Prepared Salad Mix	2.5×10^5	3.7×10^5	1.0×10^4
Prepared Salad Mix	1.8×10^5	4.1×10^5	$<5.0 \times 10^3$
Salad	1.2×10^4	1.6×10^4	$<5.0 \times 10^1$
Salad	7.0×10^3	3.0×10^4	$<5.0 \times 10^2$
Salad	8.3×10^4	5.2×10^4	$<5.0x\ 10^2$
Salad	6.0×10^4	7.0×10^4	$<5.0 \times 10^3$
Cauliflower Cheese	1.2×10^3	2.9×10^3	$<5.0 \times 10^0$
Lasagne	4.2×10^3	4.3×10^3	2.2×10^2
Lasagne	5.9×10^2	2.4×10^2	$<5.0 \times 10^0$
Chicken in Black - Bean Sauce	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Chicken Korma	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cottage Pie	2.6×10^4	2.2×10^4	5.5×10^3
Pâté	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Pâté	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Prawns	1.0×10^2	3.5×10^2	$<5.0 \times 10^1$
Prawns	$<5.0 \times 10^0$	$<5.0 \times 10^0$	$<5.0 \times 10^0$

VRBA = Violet Red Bile Agar
Petrifilm CC = Petrifilm Coliform Count Plate
Presumptive count on Petrifilm CC = count of all red colonies
Confirmed count on Petrifilm CC = count of red colonies with associated gas bubble.

TABLE 1 CONTINUED

Comparison Between Counts of Coliforms on Petrifilm and VRBA

Food	Coliforms cfu/g		
	VRBA	Petrifilm CC	
	Presumptive	Presumptive	Confirmed
Canned Tuna	5.0×10^1	9.0×10^1	$<5.0 \times 10^0$
Canned Tuna	1.0×10^2	3.0×10^2	$<5.0 \times 10^1$
Canned Tuna	1.0×10^3	1.0×10^3	$<5.0 \times 10^2$
Chilled Ham	1.4×10^5	1.3×10^5	6.0×10^4
Chilled Ham	7.9×10^9	1.8×10^8	$<5.0 \times 10^5$
Chilled Ham	2.2×10^6	2.0×10^6	$<5.0 \times 10^3$
Chilled Ham	3.1×10^6	2.9×10^6	$<5.0 \times 10^4$
Steak and Vegetable Slice	3.8×10^2	7.1×10^2	$<5.0 \times 10^0$
Steak and Vegetable Slice	5.0×10^2	9.0×10^2	$<5.0 \times 10^1$
Steak and Vegetable Slice	1.5×10^3	9.0×10^2	7.0×10^2
Steak and Vegetable Slice	5.0×10^5	3.2×10^6	$<5.0 \times 10^4$
Steak and Vegetable Slice	4.0×10^6	6.0×10^6	$<5.0 \times 10^5$
Steak and Vegetable Slice	1.2×10^2	9.0×10^1	9.0×10^1

VRBA = Violet Red Bile Agar
Petrifilm CC = Petrifilm Coliform Count Plate
Presumptive count on Petrifilm CC = count of all red colonies
Confirmed count on Petrifilm CC = count of red colonies with associated gas bubble.

TABLE 2

Comparison Between Counts of Presumptive and Confirmed Coliforms on Petrifilm and VRBA

Product	Presumptive		Confirmed	
	VRBA	Petrifilm CC	VRBA	Petrifilm CC
Chicken	3.0×10^3	TMTC	3.0×10^3	6.0×10^4
Chicken	1.4×10^4	9.9×10^3	1.4×10^4	1.3×10^3
Chicken	1.2×10^4	1.2×10^4	1.2×10^4	2.0×10^3
Chicken	4.6×10^4	4.4×10^4	3.7×10^4	1.0×10^3
Chicken	1.9×10^5	9.2×10^4	1.9×10^5	7.0×10^3
Chicken	1.9×10^5	1.1×10^5	1.9×10^5	4.0×10^4
Chicken	1.7×10^6	1.2×10^6	1.7×10^6	5.0×10^4
Chicken	2.0×10^6	1.2×10^6	2.0×10^6	1.0×10^5
Beansprouts	2.8×10^6	8.0×10^6	1.1×10^6	3.1×10^5
Beansprouts	9.2×10^6	1.2×10^7	3.7×10^6	1.3×10^6
Beansprouts	1.1×10^7	1.9×10^7	4.4×10^6	1.0×10^7
Beansprouts	1.1×10^7	1.2×10^7	6.3×10^6	6.5×10^6
Beansprouts	1.1×10^7	1.4×10^7	6.6×10^6	8.0×10^6
Beansprouts	1.7×10^7	2.2×10^7	1.4×10^7	9.9×10^6
Beansprouts	2.3×10^7	3.0×10^7	1.8×10^7	1.8×10^7
Lasagne	1.7×10^5	1.2×10^5	1.0×10^5	2.0×10^3
Lasagne	1.4×10^5	1.7×10^5	8.4×10^4	1.0×10^4
Milk	1.4×10^2	6.0×10^1	1.1×10^2	3.0×10^1
Milk	9.0×10^2	5.0×10^2	7.2×10^2	5.0×10^2
Milk	3.0×10^3	2.0×10^3	2.4×10^3	2.0×10^3

VRBA = Violet Red Bile Agar.
Presumptive Count on VRBA = Count of purple/red colonies >0.5mm diameter.
Confirmed Count on VRBA = Count of purple/red colonies >0.5mm diameter which produce acid and gas in MacConkey Broth at 37°C.
Petrifilm CC = Petrifilm Coliform Count plate.
Presumptive Count on Petrifilm CC = Count of all red colonies.
Confirmed Count on Petrifilm CC = Count of red colonies with associated gas bubble.
TMTC = Too many colonies to count at dilutions tested.

TABLE 3

Comparison Between Counts of Enterobacteriaceae on Petrifilm and VRBGA.

Food	Presumptive Enterobacteriaceae cfu/g	
	VRBGA	Petrifilm EB
Minced Beef	8.2×10^5	5.3×10^5
Minced Beef	4.4×10^6	2.9×10^6
Minced Beef	1.4×10^3	4.6×10^4
Minced Beef	1.0×10^2	3.5×10^2
Minced Pork	$<5.0 \times 10^2$	1.0×10^3
Diced Turkey	7.1×10^3	2.2×10^4
Diced Turkey	1.4×10^5	2.0×10^5
Chicken	2.9×10^4	3.3×10^4
Chicken	4.5×10^1	7.8×10^3
Chicken Burger	4.9×10^2	2.5×10^2
Chicken Burger	4.2×10^3	4.1×10^3
Sausages	5.0×10^0	4.0×10^1
Sausages	1.4×10^2	6.5×10^1
Sausages	1.6×10^3	4.3×10^3
Sausages	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Brie	1.0×10^1	7.5×10^1
Garlic Brie	2.2×10^2	2.9×10^2
Cottage Cheese and Chives	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Parmesan	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Goats' Cheese	2.5×10^3	2.4×10^3
Camembert	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cream	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cream Cake	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cream Cake	1.3×10^2	2.2×10^2
Cream Tart	4.6×10^4	2.1×10^5
Trifle	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Trifle	$<5.0 \times 10^0$	7.6×10^2
Stir Fry Veg	6.5×10^7	4.2×10^7
Stir Fry Veg	6.0×10^6	4.5×10^6
Stir Fry Veg	3.1×10^6	3.8×10^6
Stir Fry Veg	1.3×10^6	3.6×10^6
Beansprouts	7.8×10^6	3.6×10^7
Beansprouts	3.0×10^7	2.8×10^7

VRBGA = Violet Red Bile Glucose Agar
Petrifilm EB = Petrifilm Enterobacteriaceae Count Plate

TABLE 3 CONTINUED

Comparison Between Counts of Enterobacteriaceae on Petrifilm and VRBGA.

Food	Presumptive Enterobacteriaceae cfu/g	
	VRBGA	Petrifilm EB
Watercress	5.0×10^2	5.0×10^3
Watercress	1.3×10^3	6.6×10^3
Watercress	7.0×10^4	1.2×10^5
Watercress	6.1×10^5	2.1×10^6
Watercress	2.2×10^4	8.9×10^4
Lettuce	2.8×10^5	3.1×10^4
Lettuce	2.0×10^2	2.3×10^4
Mixed Veg	5.5×10^4	1.6×10^6
Carrot and Cheese	3.7×10^3	9.4×10^3
Coleslaw	1.1×10^4	9.7×10^3
Bean and Pasta Salad	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Apple and Nut Salad	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cauliflower Cheese	7.4×10^2	1.3×10^3
Lasagne	3.9×10^3	3.9×10^3
Chicken in Black Bean Sauce	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Chicken Korma	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Cottage Pie	2.5×10^4	1.0×10^4
Paté	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Paté	$<5.0 \times 10^0$	$<5.0 \times 10^0$
Prawns	1.0×10^2	3.5×10^2
Prawns	$<5.0 \times 10^0$	$<5.0 \times 10^0$

VRBGA = Violet Red Bile Glucose Agar
Petrifilm EB = Petrifilm Enterobacteriaceae Count Plate

Figure 1. Comparison between counts of presumptive coliforms on CC Petrifilm and VRBA

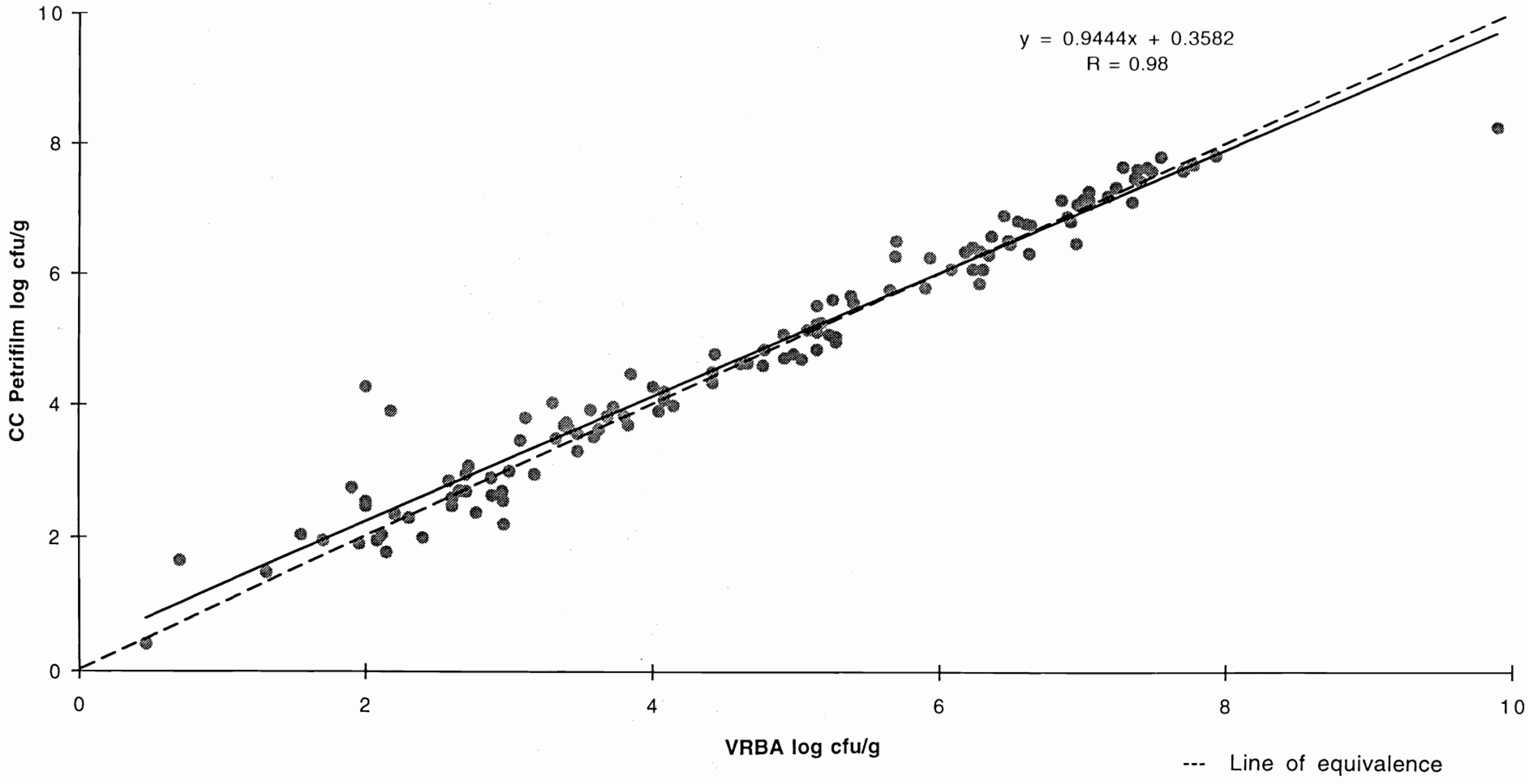


Figure 2. Comparison between counts of confirmed coliforms on CC Petrifilm and presumptive coliforms on VRBA

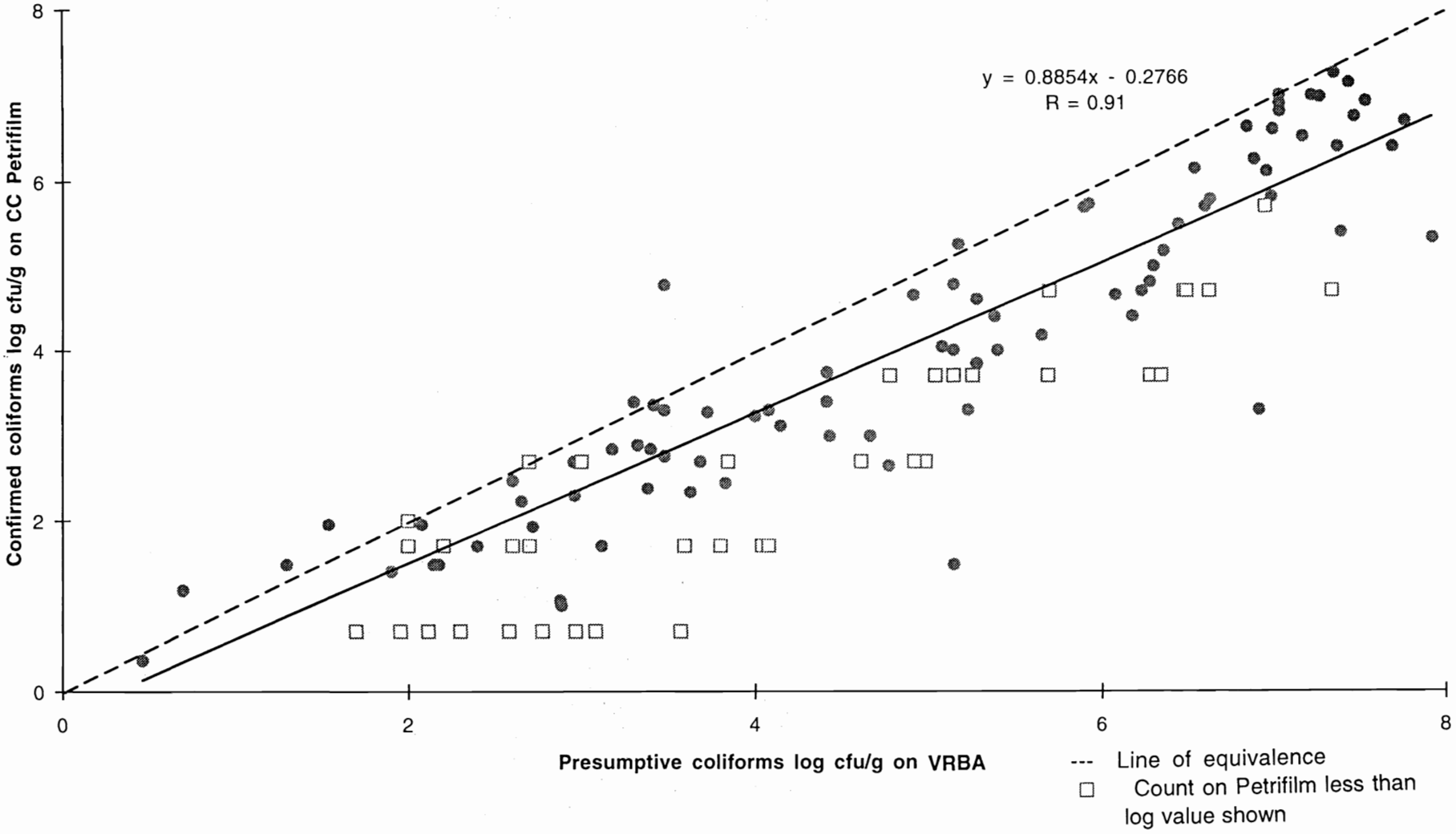


Figure 3. Comparison between counts of confirmed coliforms on CC Petrifilm and VRBA

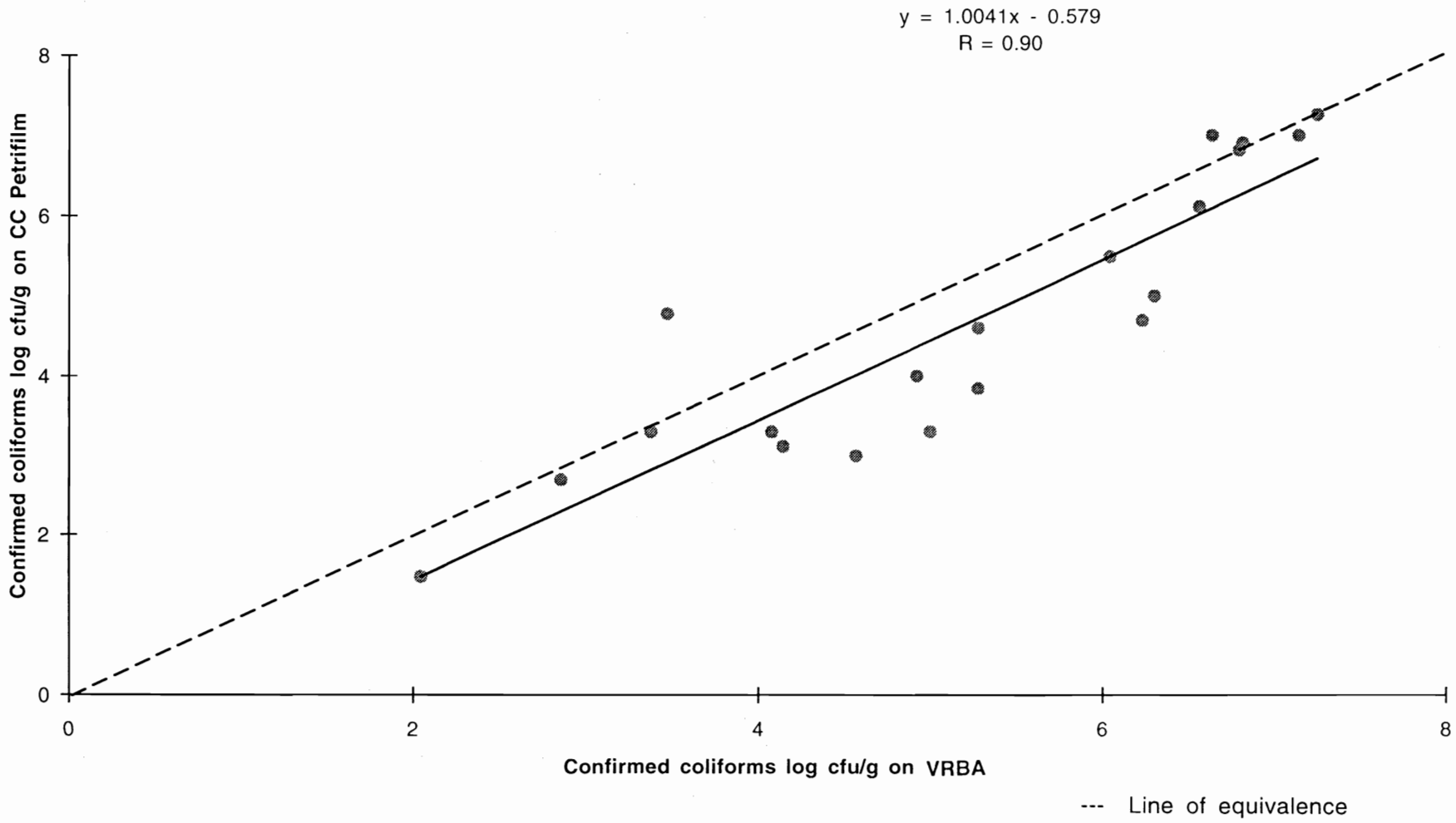
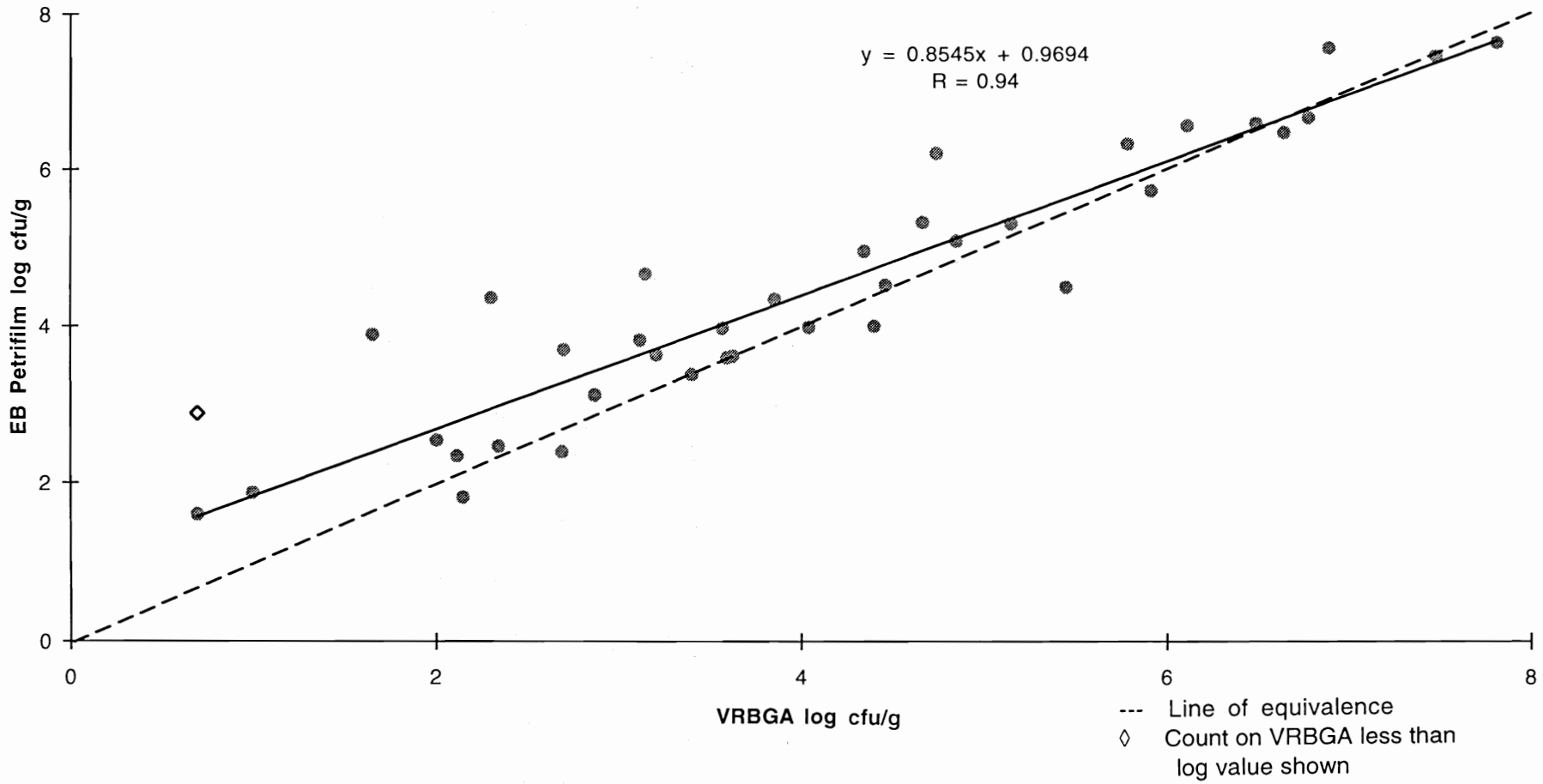


Figure 4. Comparison between counts of presumptive Enterobacteriaceae on EB Petrifilm and VRBGA



CONCLUSIONS

Petrifilm count plates can offer routine microbiology laboratories a simple, labour-saving alternative to the conventional pour-plate technique. The plates are ready to use, quick and easy to inoculate, and require minimum storage and incubator space. There is no necessity for preparation of media, and since there is no requirement for holding molten agar at a controlled temperature, no risk of heat shock to injured organisms. Petrifilm plates are available for a range of organisms, including coliforms and Enterobacteriaceae.

The Coliform Count plate has been studied and approved for use by the Association of Official Analytical Chemists (AOAC) and AFNOR. (Ginn *et al*, 1986; Curiale *et al*, 1989; and Curiale *et al,* 1991). The current study compared the Coliform Count plate (CC) and the Enterobacteriaceae Count plate (EB) with standard procedures for enumeration of these organisms in foods.

The results of counts of presumptive coliforms on Petrifilm CC compared excellently with counts obtained by the standard BS/ISO 4832 (VRBA) method in which confirmation is not routinely done. This procedure provides an alternative to the standard VRBA plate method for the enumeration of presumptive coliforms. Confirmed counts on Petrifilm compared less well with counts obtained by the BS/ISO 4832 method, and were inconclusive due to the limited data. Petrifilm CC plate can be recommended as a suitable alternative method to the ISO 4832 method for enumeration of presumptive coliforms in foods. Since most laboratories in the UK food industry only enumerate presumptive coliforms, then the use of Petrifilm as an alternative should meet most laboratories' requirements.

The Petrifilm EB plate compared well with the standard BS/ISO method for enumeration of presumptive Enterobacteriaceae and would provide an alternative to the conventional method although at low levels, the Petrifilm EB plate tended to over estimate the count compared with VRBGA. As with coliforms, most laboratories in the UK food industry only tend to enumerate presumptive Enterobacteriaceae in foods, and the Petrifilm method thus provides an alternative to the standard VRBGA plating method for presumptive Enterobacteriaceae.

ACKNOWLEDGEMENTS

Mrs. Gail Betts, Miss Alison Turner and Mr. Richard Taylor are acknowledged for their technical support.

REFERENCES

BS5763: Microbiological examination of food and animal feedings stuffs. Part 2: 1991. Enumeration of coliforms: colony count technique. (ISO 4832).

BS5763: Microbiological examination of food and animal feeding stuffs. Part 10: Enumeration of Enterobacteriaceae: colony count technique. (ISO 7402).

Curiale, M.S., Fahey, P., Fox, T.L., and McAllister, J.S. (1989). Dry rehydratable films for enumeration of coliforms and aerobic bacteria in dairy products: collaborative study. Journal of the Association of Official Analytical Chemists, **72**, (2), 312-318.

Curiale, M.S., Sons, T., McIver, D., McAllister, J.S., Halsey, B. and Roblee, D. (1991). Dry rehydratable film for enumeration of total coliforms and *Escherichia coli* in foods: collaborative study. Journal of the Association of Official Analytical Chemists, **74** (4) 635-648.

Ginn, R.E., Packard, V.S., and Fox, T.L. (1986). Enumeration of total bacteria and coliforms in milk by dry rehydratable film methods: collaborative study. Journal of the Association of Official Analytical Chemists, **69** (3), 527-531.