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A COMPARATIVE INVESTIGATION OF THE COUNTING OF BACTERIA IN MINCED MEAT BY THREE INSTITUTES

by

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1. INTRODUCTION

In the past year three institutes [1] were charged with an investigation about the growth of Salmonellae and E.coli in raw minced meat (4). This investigation was carried out in connection with former experiments (2). As our intention was to carry out this investigation independently in each laboratory, at the same time, it was necessary that the methods used were sufficiently exact and the results reproducable.
For this reason we examined the bacteriological counting methods to be employed to determine their accuracy and the possibility of obtaining the same results in the three laboratories.
During these experiments we found that, although we used the same methods, the results of the three laboratories differed fairly widely, when we counted the number of bacteria in commercial raw minced meat. To find out what were the reason(s) for the variations in the results, we carried out this investigation.

At first we counted the number of bacteria of pure cultures in liquid media and of mixtures of pure cultures in liquid media.

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After that we used a more complicated system: minced meat, prepared under septic conditions, mixed with one, two or three species of bacteria.
Finally we counted the number of bacteria in the most complicated system: commercial raw minced meat, mixed with two species of bacteria.

For the counting we used the plate count and the surface drop methods. In this way it was possible to compare these two methods. After each count we calculated the mean value and the standard deviation.
We conluded that we had a very good agreement when the standard deviations lay between 0.00 and 0.10, the agreement was good with standard deviation between 0.11 and 0.20, moderate between 0.21 and 0.30 and insufficient when the standard deviation was 0.31 and higher.

2. EXPERIMENTAL

2.1 The preparation of minced meat under aseptic conditions

From a piece of meat of about 2 kg, the outside (about 1 cm) was removed with a sterile (flamed) knife.
The inner side was out into smaller pieces on a sterile tray and minced aseptically with a sterile meat mincer.

2.2 Preparation of the samples

Before the examination, the samples were mixed. The liquid samples were mixed by shaking, the minced meat samples were aseptically mixed with a sterile fork on a sterile tray.

2.5 g Minced meat was aseptically scoured with 2.5 g sterile sand and 25 ml peptone saline solution in an sterile mortar. The content of the mortar was poured into a sterile tube. After settling for 15 minutes, the liquid layer could be used for further dilutions.

2.3 Preparation of the dilutions

Suitable dilutions (1:10) were made in the following way.
One ml was pipetted into a tube, fitted with 9 ml peptone saline solution. The content of the tube was mixed by vigorously rolling the tube between the hands.
Before 1 ml was taken for the next dilution the liquid was sucked up and down three times with a sterile 1 ml-pipette.

2.4 Methods for counting bacteria

2.4.1 Plate count method (Koch)

One ml of the dilution was poured into a Petri dish and mixed with 15 - 20 ml of the molten medium (about 50°C).

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2.5.3 VRBM-agar (violet red bile mannitol agar), according to Mossel (5)

41.5 g Difco-Bacto Violet Red Bile Agar and
10 g d-mannitol
were dissolved in 1000 ml of distilled water and heated to 100°C, The agar was used immediately.

2.5.4 Endo agar (E-agar)

40 g lactose and
10 g sodium sulphite
were dissolved in 52 ml of distilled water at 100°C for 45 minutes. After cooling 20 ml of a 10% alcoholic solution of fuchsin was added.
Ten ml of this solution was added to 400 ml of beef broth agar (2%) at 50°C. The agar was kept at this temperature till the agar was poured into the dishes.
Instead of agar of this composition Difco-Bacto Endo Agar was also used.

2.5.5 BP-agar (brilliant green phenol red agar), according to Christensen - Kauffmann.

32 ml of a phenol red solution,
1 ml of a brilliant green solution and
32 ml of a saccharose lactose solution
were mixed with 800 ml of molten beef broth agar (50°C). The agar was directly poured into the Petri dishes.

The solutions were prepared as follows:

Phenol red solution:

1 g Phenol red was dissolved in 460 ml of distilled water and 40 ml of 0.1 mol.NaOH.
The solution was kept for 2 - 3 days at 37°C, shaking occasionally.
After filtration the solution was sterilised for 20 minutes at 125°C.

Brilliant green solution:

5 g Brilliant green (according to Grübler) was dissolved in 1000 ml of distilled water, kept for some days at room temperature, shaking occasionally.

Saccharose lactose solution:

8 g Saccharose and 8 g lactose were dissolved in 32 ml of distilled water and sterilised for 45 minutes at 100°C.

Beef broth agar:

0.3% Saline and 0.2% Na_2HPO_4 2aq were added to beef broth. The pH was adjusted with NaOH at 7.4.
2% Agar was added and the beef broth agar was sterilised for 20 minutes at 120°C.

2.6 CALCULATIONS

We calculeted the logarithm of each individual count of the number of bacteria of one species in a sample. All further calculations were carried out with these logarithms.
Then we calculated the mean value of the corresponding observations.
To determine the variation of the observations, we calculated the standard deviation, when there were three or more corresponding obervations, according to:

$$\sigma = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N - 1}} \quad ; \quad \bar{x} = \frac{\sum x_i}{N}$$

σ = standard deviation
x_i = logarithm of the individual counts
$\bar{x}$ = mean value
N = number of observations

3. RESULTS

3.1 The counting of bacteria in liquid media

This part of our investigation consisted of five experiments. The first two experiments were the counting of pure cultures of S.typhi murium and E.coli. In the third and fourth experiment we counted the number of S.typhi murium and E.coli, in mixtures of these bacteria. At the last experiment of this part a third species of bacteria was introduced, namely, Enterococci. We counted independently the three species in mixtures.

All pure cultures and mixtures in beef broth were made by one of the institutes and carried to the other institutes in chilled containers (distance between the institutes about 3 km). The examination of the samples in the three institutes started at the same time.
In all experiments the surface drop method was used; in the experiments 2 and 3, two of the three institutes also used the plate count method. The results of these experiments are given in the tables 1 - 5.

The five experiments included 44 counts with three or more observations (individual counts). The following results were obtained with these counts:
- in 18 cases there was a very good agreement,
- in 10 cases there was a good agreement,
- in 9 cases there was a moderate agreement and
- in 7 cases was there insufficient agreement.

The counting of pure cultures and mixtures of bacteria in liquid media presented no difficulties.

It appears further that:

- there were only small differences between the counts of the three institutes (mean difference 0.10);
- there was only a small difference between the results with the two counting methods (mean difference 0.03);
- there were only small differences between the counts on the four different media (mean difference 0.10).

As the use of VRBM-agar had no advantage over the use of Endo agar, only the latter was employed thenceforward. With the surface drop method it was easier to see the difference between the different kinds of colonies; so we used only this method from then on.

3.2 The counting of bacteria in minced meat, prepared under aseptic conditions.

Before we counted the number of bacteria in commercial raw minced meat we used minced meat prepared under aseptic conditions in one of the institutes. In this way we limited the influence of the many bacteria normally present in commercial minced meat. The second part of our investigation consisted of three experiments.

In the first experiment the minced meat was mixed with pure cultures of S.typhi murium, in the second experiment E.coli was also added and in the third experiment a third species of bacteria was introduced: Staph.albus. All these species of bacteria were counted independently at the same time. The results of these experiments are given in the tables 6-8.
These three experiments included 29 counts with three or more observations (individual counts). With these counts we obtained the following results:

- in 8 cases there was a very good agreement,
- in 16 cases there was a good agreement,
- in 2 cases there as a moderate agreement and
- in 3 cases there was insufficient agreement.

The counting of mixtures of pure cultures of bacteria with minced meat, prepared under aseptic conditions, gave no difficulties.

3.3 The counting of bacteria in commercial raw minced meat.

The third part of our investigation was the most complicated. The bacteria present in commercial minced meat (bought in a normal butcher's shop in Utrecht) were introduced.

3.3.1 Counting without precautions

In the first and second experiment of this part we had taken no special precautions. In the first experiment the minced meat was mixed with a pure culture of S.typhi murium or E.coli; in the second experiment the minced meat was mixed with mixtures of these two species of bacteria. The results of these experiments are given in the tables 9 and 10. The two experiments included 23 counts with three or more observations (individual counts). With these counts we obtained the following results:

- in only 2 cases there was a very good agreement,
- in 13 cases there was a good agreement,
- in 2 cases there was a moderate agreement and
- in 6 cases (26%) there was insufficient agreement.

It appears that counting the number of bacteria in commercial minced meat is surrounded by more difficulties than counting the number of bacteria in liquid media and in minced meat prepared under aseptic conditions.

3.3.2 Counting in one room with the same media

It seems that it was the way the co-workers of the three institutes carried out the work that caused these inferior results.
We decided therefore to carry out the next experiments in one laboratory-room. In this way it was possible for the co-workers of the three institutes to make an acurate comparising their respective ways of going about the work. Also the same media for the countings were used.
The result of this experiment is given in table 11. It appears that now there was a very good agreement. In this experiment we again used the two counting methods. We obtained a very good agreement between the surface drop method and the plate count method.

The following experiments were all carried out in the same room. In addition we decided that the countings should be done twice . The results are given in the tables 12 and 13:

- in 5 cases there was a very good agreement,
- in 13 cases there was a good agreement,
- in no cases was there a moderate agreement and
- in only 2 cases was there insufficient agreement.

The counting of the number of bacteria in commercial raw minced meat was possible, provided that counting methods were the same in all details and the same media were used.

In our further investigations we counted the number of bacteria in our laboratories, but we always used the same media and a completely indentical counting method. The agreement was usually good or very good.

4. DISCUSSION

It appears, from our investigation, that the counting of the number of bacteria in one sample by different institutes did not always give the same results.

So long as only samples, with a few known species of bacteria, were examined, there were not many difficulties. With the counting of the number of bacteria in pure cultures and mixtures of pure cultures in liquid media and in aseptically prepared minced meat, mixed with one, two or three species of bacteria, we always obtained a good agreement between the individual counts.
But if, as in commercial raw minced meat, a great number of unknown bacteria was introduced, the counting of the number of Salmonellae and E.coli by three laboratories independently was not so easy. In the first two experiments with commercial minced meat we got inferior results compared with former experiments.
When we carried out the next experiments in one room, it appeared that there were small differences between the techniques of the co-workers of the three institutes.
There were small differences in: scouring the samples, mixing the dilutions, placing the drops on the surface of the Petri dishes and the preparation of the media.

When we used exactly the same methods, as described in chapter 2, we obtained better results.

From our investigation we drew the following conclusions:

When a bacteriological investigation is carried out by two or more institutes, or when an investigation of an institute is repeated or completed by another institute, it is necessary that both experiments, with special regard to the counting methods and the media, be carried out in the same way in all parts.
Only in this way they can obtain comparable results.

5. REFERENCES

(1) Badger, E.H.M. and Pankhurst, E.S.
Experiments on the accuracy of surface drop bacterial counts.
J.Appl. Bact. 23 (1960) 28-36.

(2) Krol, B.and Moerman, P.C.
De invloed van sulfiet op de kleur en de bacteriologische gesteldheid van gehakt. (The influence of sulphite on colour and keeping quality of minced meat).
Conserva 8 (1959/60) 1-11

(3) Miles, A.A. and Misra, S.S.
The estimation of the bactericidal power of the blood.
J.Hyg. 38 (1938) 732.

(4) Moerman, P.C.
De invloed van natriumsulfiet op de groei van Salmonella en E.coli in rauw handelsgehakt.
(The influence of sodium sulphite on the growth of Salmonella and E.coli in raw commercial minced meat).
Report of a working group of the Research Group for Meat and Meat Products T.N.O., 1962.

(5) Mossel, D.A.A.
Appl. Microbiol. 5 (1955) 379-381.

Summary

A comparative investigation on the counting of Salmonellae and E.coli in the same samples was carried out by three institutes.
Good agreement was obtained with the counting of bacteria in liquid samples (pure cultures) and in minced meat, prepared under aseptic conditions and mixed with pure cultures of bacteria.
Counting the number of bacteria in commercial minced meat gave good results only when all details of the counting method were carried out in the same way, and when the media used were prepared exactly in the same way. Complete similarity in the methods was obtained by working in one laboratory room.

Resumé

Une recherche comparative concernant le dénombrement de Salmonellae et E.coli dans les mêmes échantillons fut réalisée par trois instituts.
Une bonne analogie était obtenue avec le dénombrement des bactéries dans les échantillons liquides (boillons de culture) et dans viandes hâchées, préparées dans des circonstances aseptiques et mêlées avec bouillons de culture.
Le dénombrement des bactéries dans viandes hâchées commerciales donnent seulement des bons résultats, si tous les détails de la méthode pour le dénombrement étaient fait de la même manière et les media préparé à la même manière, étaient utilisé. Une analogie complète fut obtenu seulement, après travailler dans une salle de laboratoire.

Zusammenfassung

In drei verschiedenen Instituten wurden vergleichende Keimzählungen mit Salmonellae und E.coli ausgeführt.
Eine gute Übereinstimmung wurde bei Keimzählungen von Reinkulturen in flüssigen Medien arziehlt und in Hackfleisch-Proben, die unter aseptischen Umständen bereitet, mit Reinkulturen gemischt wurden.
Keimzählungen in normalem Verkauf-Hackfleisch führten nur dann zu vergleichbaren Resultaten wenn die Technik der Zählmethoden mit grösster Genauigkeit auf einander abgestimmt und die verwendeten Nährboden in ganz gleicher Weise hergestellt wurden. Eine gute Übereinstimmung wurde auszerdem erziehlt wenn die Untersuchenden in einem Raume arbeitetten.

Table 1. Counting of the number of bacteria, in 3 samples of pure cultures of S.typhi murium and E.coli, by the surface drop method on different media by three institutes (A, B and C).

sample	organism	medium	log counts/ml, counted by			mean value (log)
			A	B	C	
1	S.typhi murium	E-a.	-	7.75	7.62	
		BP-a.	7.40	7.70	7.51	7.54 ± 0.20
		TGY-a.	7.23	7.78	7.57	
		VRBM-a.	-	-	7.28	
2	S.typhi murium	E-a.	8.78	8.66	8.99	
		BP-a.	9.04	-	8.92	8.91 ± 0,14
		TGY-a.	8.95	8.86	8.96	
		VRBM-a.	-	-	9.02	
3	E.coli	E-a.	8.85	7.72	7.40	
		TGY-a.	8.85	7.82	7.18	7.86 ± 0.71
		VRBM-a.	-	-	7.23	

Table 2. Counting of the number of bacteria, in 4 samples of pure cultures of S. typhi murium and E. coli, by the surface drop method and the plate count method on different media by three institutes (A, B and C).

sample	organism	method	medium	log counts/ml, counted by A	B	C	mean value (log)
21	S. typhi murium	surface drop	E-a.	-	8.60	8.15	8.32 ± 0,25
			BP-a.	8.11	8.51	8.30	
			TGY-a.	8.30	8.75	8.00	
			VRBM-a.	-	-	8.18	
		plate count	E-a.	-	-	8.11	8.08 ± 0,20
			BP-a.	-	-	8.23	
			TGY-a.	8.20	-	8.04	
			VRBM-a.	8.20	-	7.70	
22	S. typhi murium	surface drop	E-a.	8.73	8.90	8.85	8.90 ± 0,11
			BP-a.	8.83	8.92	9.08	
			TGY-a.	8.78	8.92	9.00	
			VRBM-a.	-	-	9.00	
		plate count	E-a.	-	-	8.88	8.84 ± 0.09
			BP-a.	-	-	8.95	
			TGY-a.	8.70	-	8.90	
			VRBM-a.	8.70	-	8.89	
23	E. coli	surface drop	E-a.	8.70	8.66	8.04	8.45 ± 0.33
			TGY-a.	8.78	8.36	8.08	
		plate count	E-a.	-	-	7.28	7.80 ± 0.75
			TGY-a.	8.78	-	7.46	
			VRBM-a.	8.78	-	6.70	
24	E. coli	surface drop	E-a.	8.85	8.87	9.00	8.90 ± 0.06
			TGY-a.	8.90	8.86	8.95	
			VRBM-a.	-	-	8.95	
		plate count	E-a.	-	-	9.00	8.92 ± 0.07
			TGY-a.	8.85	-	8.96	
			VRBM-a.	8.85	-	8.95	

Table 3 Counting of the number of bacteria, in 3 samples of mixed cultures of S.typhi murium and E.coli, by the surface drop method and the plate count method on different media by three institutes (A, B and C).

sample	method	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
31	surface drop	BP-a.	Salm.	-	8.57	8.45)	8.51 ± 0.10
		E-a.	Salm.	-	8.63	8.40)	
		E-a.	E.coli	-	6.95	7.04	7.00
		VRBM-a.	Salm. + E.coli	-	-	8.43)	
		TGY-a.	Salm. + E.coli	-	8.65	8.52)	8.53 ± 0.11
	plate count	BP-a.	Salm.	8.08	-	8.66)	8.33 ± 0.29
		E-a.	Salm.	8.26	-	-)	
		E-a.	Salm. + E.coli	-	-	8.38)	
		VRBM-a.	Salm. + E.coli	8.08	-	8.14)	8.25 ± 0.18
		TGY-a.	Salm. + E.coli	8.14	-	8.49)	
32	surface drop	BP-a.	Salm.	-	7.04	6.95)	7.00 ± 0.05
		E-a.	Salm.	-	7.00	-)	
		E-a.	E.coli	-	8.72	8.54	8.63
		VRBM-a.	Salm. + E.coli	-	-	8.48)	
		TGY-a.	Salm. + E.coli	-	8.79	8.23)	8.50 ± 0.28
	plate count	BP-a.	Salm.	8.97	-	8.60)	8.85 ± 0.22
		E-a.	Salm.	8.98	-	-)	
		E-a.	E.coli	9.04	-	-	-
		E-a.	Salm. + E.coli	-	-	8.38)	
		VRBM-a.	Salm. + E.coli	8.87	-	8.40)	8.63 ± 0.29
		TGY-a.	Salm. + E.coli	9.00	-	8.48)	
33	surface drop	BP-a.	Salm.	-	7.11	7.04)	7.08 ± 0.05
		E-a.	Salm.	-	7.14	7.04)	
		E-a.	E.coli	-	6.82	6.74	6.78
		VRBM-a.	Salm. + E.coli	-	-	7.28)	
		TGY-a.	Salm. + E.coli	-	7.34	7.23)	7.28 ± 0.05
	plate count	BP-a.	Salm.	7.14	-	7.11)	7.00 ± 0.21
		E-a.	Salm.	6.76	-	-)	
		E-a.	E.coli	7.04	-	-	-
		E-a.	Salm. + E.coli	-	-	7.14)	
		VRBM-a.	Salm. + E.coli	6.20	-	6.81)	6.94 ± 0.48
		TGY-a.	Salm. + E.coli	7.46	-	7.11)	

Table 4. Counting of the number of bacteria, in 5 samples of mixed cultures of S.typhi murium and E.coli, by the surface drop method on different media by three institues (A, B and C).

sample	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
41	BP-a.	Salm.	7.23	7.43	7.41)	7.39 ± 0.09
	E-a.	Salm.	7.36	7.34	7.48)	
	E-a.	E.coli	7.90	7.85	7.70	7.82 ± 0.10
	VRBM-a.	Salm. + E.coli	-	-	7.95)	
	TGY-a.	Salm. + E.coli	8.00	8.08	8.00)	8.01 ± 0.06
42	BP-a.	Salm.	6.72	7.14	7.30)	7.25 ± 0.33
	E-a.	Salm.	7.48	7.18	7.70)	
	E-a.	E.coli	7.62	7.79	7.78	7.73 ± 0.10
	VRBM-a.	Salm. + E.coli	-	-	7.87)	
	TGY-a.	Salm. + E.coli	7.90	7.85	7.88)	7.88 ± 0.02
43	BP-a.	Salm.	8.36	8.62	8.60)	8.58 ± 0.10
	E-a.	Salm.	8.60	8.60	8.60)	
	E-a.	E.coli	7.20	7.63	7.48	7.44 ± 0.22
	VRBM-a.	Salm. + E.coli	-	-	8.61)	
	TGY-a.	Salm. + E.coli	8.54	8.70	8.66)	8.63 ± 0.07
44	BP-a.	Salm.	6.95	7.34	7.36)	7.50 ± 0.45
	E-a.	Salm.	8.30	7.48	7.60)	
	E-a.	E.coli	8.80	8.63	8.82	8.75 ± 0.09
	VRBM-a.	Salm. + E.coli	-	-	8.89)	
	TGY-a.	Salm. + E.coli	8.64	8.74	8.80)	8.77 ± 0.10
45	BP-a.	Salm.	8.04	8.18	8.40)	8.24 ± 0.16
	E-a.	Salm.	8.12	8.20	8.48)	
	E-a.	E.coli	9.04	8.86	8.78	8.89 ± 0.13
	VRBM-a.	Salm. + E.coli	-	-	8.86)	
	TGY-a.	Salm. + E.coli	9.04	8.95	8.93)	8.95 ± 0.07

Table 5. Counting of the number of bacteria, in 2 samples of mixed cultures of S.typhi murium, E.coli and Enterococci, by the surface drop method on different media by three institutes (A, B and C).

sample	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
51	BP-a.	Salm.	6.67	7.34	7.56)	7.17 ± 0.31
	E-a.	Salm.	7.23	7.23	7.00)	
	E-a.	E.coli	8.08	8.14	8.00	8.07 ± 0.08
	VRBM-a.	Salm. + E.coli	-	-	8.20	-
	TGY-a.	Total count	8.53	8.32	8.30	8.38 ± 0.12
	TGY-a.	Enterococci	-	7.74	7.78	7.76
52	BP-a.	Salm.	8.18	8.26	8.08)	8.32 ± 0.22
	E-a.	Salm.	8.40	8.30	8.70)	
	E-a.	E.coli	9.18	8.74	8.90	8.94 ± 0.22
	VRBM-a.	Salm. + E.coli	-	-	8.92	-
	TGY-a.	Total count	9.20	8.90	8.91	9.00 ± 0.17
	TGY-a.	Enterococci	-	7.65	-	-

Table 6. Counting of the number of bacteria, in 2 samples of minced meat, prepared under aseptic conditions and mixed with pure cultures of S. typhi murium, using the surface drop method by three institutes (A, B and C).

sample	medium	log counts/ml, counted by			mean value (log)
		A	B	C	
61	BP-a.	3.92	3.62	3.85	3.76 ± 0.13
	E-a.	3.92	3.58	3.75	
	TGY-a.	3.80	3.65	5.38 1)	
62	BP-a.	6.63	6.57	6.65	6.65 ± 0.05
	E-a.	6.66	6.71	6.63	
	TGY-a.	6.60	6.71	6.71	

1) This number is leaved out of account.

Table 7. Counting of the number of bacteria, in 2 samples of minced meat, prepared under aseptic conditions and mixed with pure cultures of S. typhi murium and E. coli, using the surface drop method by three institutes (A, B and C).

sample	medium	organisms counted	log counts/ml, counted by			mean value (log)
			A	B	C	
71	BP-a.	Salm.	6.49	6.45	6.58)	6.60 ± 0.12
	E-a.	Salm.	6.76	6.56	6.73)	
	E-a.	E. coli	5.08	5.20	4.81	5.03 ± 0.19
	TGY-a.	Salm. + E. coli	6.74	6.61	6.77	6.71 ± 0.09
72	BP-a.	Salm.	3.71	3.56	3.75	3.67 ± 0.10
	E-a.	E. coli	6.68	6.56	6.76	6.66 ± 0.10
	TGY-a.	Salm. + E. coli	6.51	6.51	6.84	6.69 ± 0.17

Table 8 Counting by three institutes (A, B and C), of the number of bacteria, in 6 samples of minced meat, prepared under aseptic conditions and mixed with pure cultures of S.typhi murium, E.coli and Staph.albus, using the surface drop method.

sample	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
81	BP-a.	Salm.	4.11	3.82	4.04)	3.98 ± 0.13
	E-a.	Salm.	4.11	3.83	3.98)	
	E-a.	E.coli	2.85	2.70	2.78	2.78 ± 0.07
	TGY-a.	Salm. + E.coli	-	3.99	4.04	4.02
	TGY-a.	St.albus	-	2.95	3.08	3.02
	TGY-a.	total counts	4.17	-	-	-
82	BP-a.	Salm.	4.48	4.54	4.36)	4.60 ± 0.17
	E-a.	Salm.	4.72	4.65	4.85)	
	E-a.	E.coli	-	-	1.60	-
	TGY-a.	Salm. + E.coli	4.95	4.64	4.67	4.75 ± 0.17
	TGY-a.	St.albus	6.08	5.78	5.93	5.93 ± 0.15
83	BP-a.	Salm.	4.86	4.80	4.68)	4.77 ± 0.08
	E-a.	Salm.	4.79	4.81	4.69)	
	E-a.	E.coli	3.78	3.64	3.81	3.74 ± 0.09
	TGY-a.	Salm. + E.coli	5.00	5.00	4.65	4.88 ± 0.21
	TGY-a.	St.albus	6.18	6.04	5.79	6.01 ± 0.20
84	BP-a.	Salm.	3.78	3.63	3.91)	3.78 ± 0.11
	E-a.	Salm.	3.85	3.67	3.82)	
	E-a.	E.coli	3.78	3.68	3.88	3.78 ± 0.10
	TGY-a.	Salm. + E.coli	4.30	4.04	4.18	4.17 ± 0.13
	TGY-a.	St.albus	3.14	2.78	2.86	2.93 ± 0.19
85	BP-a.	Salm.	4.90	4.70	4.99)	4.83 ± 0.14
	E-a.	Salm.	4.90	4.63	4.89)	
	E-a.	E.coli	3.28	2.30	2.84	2.81 ± 0.49
	TGY-a.	Salm. + E.coli	5.00	4.67	4.90	4.86 ± 0.19
	TGY-a.	St.albus	6.11	5.78	5.85	5.91 ± 0.17
86	BP-a.	Salm.	2.95	2.60	2.95)	2.95 ± 0.23
	E-a.	Salm.	-	3.18	-)	
	E-a.	E.coli	4.90	4.23	4.95	4.69 ± 0.40
	TGY-a.	Salm. + E.coli	4.85	4.26	4.88	4.66 ± 0.36
	TGY-a.	St.albus	6.04	5.78	5.88	5.90 ± 0.13

Table 9 Counting, by three institutes (A, B and C), of the number of bacteria, in 2 samples of commercial minced meat and in 4 samples of commercial minced meat, mixed with pure cultures of S.typhi murium and E.coli, using the surface drop method.

sample	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
91	E-a.	E.coli	-	-	3.51	-
	TGY-a.	total counts	6.11	6.54	5.51	6.05 ± 0.48
92	BP-a.	Salm.	6.23	6.76	6.23)	6.57 ± 0.31
	E-a.	Salm.	6.92	6.85	6.45)	
	E-a.	E.coli	-	-	3.98	-
	TGY-a.	total counts	6.97	6.85	6.43	6.75 ± 0.28
93	BP-a.	Salm.	6.79	6.76	6.60)	6.78 ± 0.11
	E-a.	Salm.	6.90	6.85	6.78)	
	E-a.	E.coli	6.11	5.88	5.85	5.95 ± 0.15
	TGY-a.	total counts	7.04	6.88	6.73	6.88 ± 0.16
94	BP-a.	Salm.	3.48	3.34	3.54	3.45 ± 0.10
	E-a.	Salm. + E.coli	4.45	-	4.20	4.33
	E-a.	E.coli	-	3.60	2.67	3.14
	TGY-a.	total counts	5.66	6.63	5.53	5.94 ± 0.60
95	BP-a.	Salm.	6.85	6.48	7.08)	6.89 ± 0.25
	E-a.	Salm.	6.95	-	7.08)	
	E-a.	E.coli	5.34	4.18	4.92	4.81 ± 0.47
	TGY-a.	total counts	7.00	6.93	6.96	6.96 ± 0.04
96	BP-a.	Salm.	4.85	4.65	4.86	4.79 ± 0.11
	E-a.	E.coli	7.00	6.76	6.89	6.88 ± 0.11
	TGY-a.	total counts	7.08	6.85	6.79	6.90 ± 0.16

Table 10 Counting by three institutes (A, B and C), of the number of bacteria, in 1 sample of commercial minced meat and 2 samples of commercial minced meat, mixed with pure cultures of S. typhi murium and E. coli, using the surface drop method.

sample	medium	organisms counted	log counts/ml, counted by A	B	C	mean value (log)
101	BP-a.	Salm.	2.51 2.56	-	2.30 2.40	2.44 ± 0.12
	E-a.	Salm. + E. coli	4.95 5.00	5.93 5.96	4.66 4.85	5.23 ± 0.39
	TGY-a.	total counts	5.85 5.95	6.20 6.23	5.86 5.89	6.00 ± 0.18
102	BP-a.	Salm.	6.85 6.78	6.57 6.54	6.76 6.82	6.71 ± 0.11
	E-a.	Salm.	6.60 6.60	6.74 6.62	6.81 6.78	
	E-a.	E. coli	4.68 4.36	4.48 4.48	4.66 4.64	4.55 ± 0.15
	TGY-a.	total counts	6.82 6.82	6.54 6.74	6.70 6.77	6.73 ± 0.11
103	BP-a.	Salm.	4.78 5.38	4.65 4.48	4.68 4.76	4.86 ± 0.31
	E-a.	Salm.	4.85 5.40	4.80 -	- -	
	E-a.	E. coli	7.11 6.78	6.88 6.94	6.72 6.58	6.83 ± 0.18
	TGY-a.	total counts	7.08 6.90	6.85 6.99	6.78 6.67	6.88 ± 0.14

Table 11 Using one room, the co-workers (A, B and C) of three institutes counted the number of bacteria in 2 samples of commercial minced meat, mixed with a pure culture of E.coli. The surface drop and the plate count methods were used. The bacteria were counted on endo agar.

sample	method	log counts/ml, counted by			mean value (log)
		A	B	C	
111	surface drop	7.08	6.95	6.90	7.03 ± 0.07
		7.11	7.00	6.95	
		7.11	7.08	7.08	
		7.11	6.95	6.95	
		7.08	7.04	6.95	
		7.11	7.08	6.95	
	plate count	7.04	6.95	6.90	7.01 ± 0.06
		7.04	7.00	6.95	
		7.11	7.04	7.04	
		7.11	6.95	7.00	
		7.04	7.00	7.04	
		7.08	7.00	6.90	
112	surface drop	7.00	6.85	6.70	6.88 ± 0.09
		6.95	6.90	6.85	
		6.85	6.95	6.85	
		6.90	6.85	6.70	
		6.95	6.85	6.70	
		7.00	6.90	7.00	
	plate count	6.78	6.78	6.85	6.87 ± 0.08
		6.95	6.85	6.90	
		6.70	6.85	7.00	
		6.85	6.85	6.90	
		6.78	6.85	6.95	
		7.00	6.85	7.00	

Table 12 Counting, by co-workers (A,B and C) of three institutes, in one room,of the number of bacteria in 8 samples of commercial minced meat, mixed with a pure culture of E.coli, using the surface drop method. The bacteria were counted on endo agar.

sample	log counts/ml, counted by			mean value (10⁹)
	A	B	C	
121	8.18 8.00	8.00 8.00	8.14 8.32	8.11 ± 0.13
122	8.57 8.58	8.36 8.30	8.32 8.52	8.44 ± 0.13
123	9.04 9.08	9.18 9.26	8.95 9.04	9.08 ± 11
124	8.68 8.67	8.70 9.14	8.66 8.76	8.77 ± 0.18
125	8.58 8.65	8.48 8.48	8.66 8.53	8.56 ± 0.08
126	6.32 6.40	6.11 6.11	6.04 6.08	6.18 ± 0.14
127	7.20 7.32	7.08 7.08	7.00 6.95	7.11 ± 0.13
128	7.65 7.68	7.60 7.60	7.56 7.46	7.59 ± 0.08

Table 13 Counting, by co-workers (A, B and C), of three institutes, in one room, of the number of bacteria in 6 samples of commercial minced meat, mixed with a pure culture of E.coli, using the surface drop method.

sample	medium	organisms counted	log counts/ml, counted by			mean value (log)
			A	B	C	
131	E-a.	E.coli	2.48 2.75	4.11 4.20	3.14 3.38	3.34 ± 0.70
	TGY-a.	total counts	5.00 -	5.23 5.11	5.75 5.79	5.38 ± 0.37
132	E-a.	E.coli	4.51 4.57	4.54 4.60	4.53 4.58	4.56 ± 0.03
	TGY-a.	total counts	5.38 5.46	5.54 5.52	5.77 5.51	5.53 ± 0.13
133	E-a	E.coli	6.90 6.78	6.76 6.65	6.65 6.48	6.70 ± 0.14
	TGY-a.	total counts	6.90 7.04	6.76 6.70	6.59 6.58	6.76 ± 0.17
134	E-a.	E.coli	2.59 2.71	2.70 2.85	2.93 3.00	2.80 ± 0,15
	TGY-a.	total counts	4.53 4.64	4.64 4.82	4.84 4.83	4.72 ± 0.13
135	E-a.	E.coli	4.72 4.74	4.66 4.73	4.61 4.59	4.68 ± 0.07
	TGY-a.	total counts	4.88 4.84	4.85 4.91	4.95 5.18	4.94 ± 0.14
136	E-a.	E.coli	6.85 6.85	6.62 6.57	6.65 6.68	6.70 ± 0.12
	TGY-a.	total counts	6.85 6.85	6.70 -	6.63 6.72	6.75 ± 0.09