4 :14

Direct Epifluorescent Filter Technique as a rapid method in microbiological quality assuarance of the meat products - experience in practice

J.KYLLAROVA and J.LUKAŠOVA

Meat Research Institute, Palackeho 1-3, 612 00 Brno, CSFR
University of Veterinary Medicine, Palackeho 1-3, 612 00 Brno, CSFR

SUMMARY: Direct Epifluorescent Filter Technique /DEFT/ has not been used in food microbiology in Czechoslovakia yet. The DEFT has been tested on samples of cured and raw ground meat, seasoned minced meat and some heat treated meat products /salami, "liver paté salami"/.

The results obtained from the DEFT within one hour were satisfying. Good agreement between DEFT and standard plate count /SPC/ was found for these products within the range of 10^4 to 10^8 g^{-1}. The correlation coefficient was 0,87 /raw ground meat/, 0,82 /cured meat/, 0,86 /seasoned minced meat/, 0,81 /salami/ and 0,79 /"liver paté salami"/.

The conclusion was made that DEFT could be used as a screening method to ensure the short period of microbiological examination of raw meat and some meat products.

INTRODUCTION: Food microbiology has been greatly influenced by the rapid microbiologic methods. One of them is Direct Epifluorescent Filter Technique - DEFT. This method connes advantages of the direct microscopy with the membrane filter technic. The DEFT was initia designed for dairy applications /PETTIPHER et al.,1980/. Later the DEFT was used for raw meat /BAUMGART et al.,1981/, vegetables, fishes and frozen meat /PETTIPHER et al.,1982/. JAKOBSEN and QUIST /1984/ applicated the DEFT for minced meat and meat products. The base of the DEFT consists in the fluorochromic stain bind on RNA and DNA of microbes' cells fluorescing against a dark backround.

MATERIALS and METHODS: Four categories of meat products were investigated 1.raw groun meat, 2.cured meat, 3.seasoned minced meat, 4.heat treated meat products as salami and "liver paté salami".

The method recommended by JAKOBSEN and QUIST /1984/ was used. To meat samples of 10 gr 90 ml of dilution medium /1% pepton, 0,5% NaCl/ were added, and the samples were homogeni for 2 min. in a"STOMACHER". The homogenous samples were examined by standard cultivation technique with 48 hour incubation at 30°C on the meat pepton agar /norm ČSN 56 00 83/.

After 30 min. standing for settlement of larger particles were the same homogenous samples used for direct epifluorescent filter technique. 5 ml of the suspension were filtered through the sick sterile paper filter. To 2 ml of the filtered suspension in a sterile test tube, 2 ml 0,5% Triton x-100 and 0,5 ml 0,25% trypsin were added. The mixt was incubated in a waterbath at 50°C for 10 min. and then filtered through a SYNPOR membr filter /0,4 size/ mounted to a vacuum water pump.

After filtration the membrane was overlayed with 2,5 ml acridine orange. After standi for 2 min. vacuum was re-applied in order to filter the stain. Filter was washed with 2,5 citrate buffer/ pH 3,0 / followed by 2,5 ml iso-propanol to decolorize the filter.

The stained membrane was airdried, mounted in a canadian balsam under a cover slip and examined by means of an epifluorescence microscope /JENAMED/.

The number of fluorescent microbes were counted at a magnification of 1000. The number of microbes per gram was calculated by multiplying the average number of bacteria counted per microscope field by the microscope factor. We counted all orange and green light microbes.

For every ivestigated samples group was calculated the correlation coefficient.

RESULTS and DISCUSSION: Seventy samples were tested at all. The most of microbes fluoresced orange against a dark backround. The green fluorescent microbes occured rarely All of microbes were easy to distinguish from stain remainders or slight quantity of tiss

The results received by the DEFT were very similar to the results obtained by the conventional method.

The standard plate count for raw ground meat was 10^4-10^9 g^{-1} , the DEFT 10^5- 10^9 g^{-1} /r= 0,87/. The standard plate count for cured meat was 10^6- 10^9 g^{-1}, the DEFT 10^6-10^8 g^{-1} /r= 0,82/. The standard plate count for seasoned minced meat was 10^5- $10^8 g^{-1}$, the DEFT 10^5- $10^7 g^{-1}$ /r= 0,86/. The standard plate count for "liver paté salami"was 10^4- $10^8 g^{-1}$, the DEFT 10^5- $10^7 g^{-1}$ /r= 0,79/. The standard plate count for salami was 10^4- $10^8 g^{-1}$, the DEFT 10^5- $10^8 g^{-1}$ /r= 0,81/.

The statistical evaluation of obtained results proved the narrow relation between the DEFT and SPC / Fig. 1 - 3 /. The best correlation with SPC were found in space of 10^5 to 10^7 microbes per gram what is basicaly identic with literature citations /JAKOBSEN and QUIST, 1984 , BOISEN, 1984/. Counting 10^8 g^{-1} and more microbes is very difficult for reason of their great quantity per microscope field / sometimes they are in many layers /.

The differences between results SPC and DEFT weren't statistically indicative with exception group samples of cured meat / Fig.4 / It could be due probably to the great microbes quantity / 10^8 g^{-1} / in many samples.

The DEFT make possible to know the composition of food microflora /cocci, rods, yeasts etc./. In the investigated samples rods predominated, there were rarely sporulate microbes yeasts and cocci. The microbe differentiation based only on their morphology is not common valuable / RODRIGUES and KROLL 1988 /.

The disadvantage of the DEFT consists in the fact that the fluorescent stain colourizes the active and death microbes' cells. Citation conforming with HOFFIE et al./1977/ the active microbes have orange fluorescing and inactive microbes are fluorescing in the green colour which depends on the bind of acridine orange on DNA /ortochromatic fluorescence - green / and on RNA /metachromatic fluorescence - orange /. However the observation of PETTIPHER et al. /1980, 1982/ and RODRIGUES et al. /1986/ on the activ cultures approve that somewhere apparently inactive cells fluorescing green can be active.

Modification of the method in this way brought RODRIGUES and KROLL /1988/. The improvement consists in cultivation the membrane filter with microbes' cells on the cover of nutrient agar and 3 hours incubation at 30°C. Then the membrane filter colorization with acridine orange and counting microcolonies follow. The living colony were colourized by orange and they were very good recognized from not growing microbes' clumps. The results were available during a working day.

CONCLUSION : The DEFT appears as a valuable method for microbiological screening of meat and some meat products. The DEFT does not require a large working place and gives reproducible results in short time. The samples preparation and the standard plate count results evaluation needs 3 days minimum. Comparable results of the DEFT with standard plate count were obtained in application of the DEFT after 60 minutes.

REFERENCES : BAUMGART,J., SCHIERHOLZ,B. and HUY,CH./1981/: Membranefilter-Mikrokolonie -Fluorescenz-Methode zum Schnellnachweis des Oberflächenkeimgehaltes von Frischfleisch. Fleischwirtschaft 61, pp.726,729-730.

BOISEN,F. /1984/: Practical experience with a rapid microbiological method using the DEFT/ Direct Epifluorescence Filter Technique/ procedure. Dansk Veterinaertidsskrift 66, pp. 1030-1037.

ČSN 56 00 83 /norm//1987/: Stanovení celkového počtu mezofilních aerobních a fakultativně anaerobních mikroorganismů plotnovou metodou.

HOFFIE,J.E.,DALEY,R.S. and JASPER,S. /1977/: Use of nucleopore filters for counting bacteria by fluorescence microscopy.Appl. Enviromn. Microbiol.33, pp, 1225-1228.

JAKOBSEN,M. and QUIST,S./1984/:The direct epifluorescence filter technik as a rapid method for microbiological analysis of meat products. Dansk Veterinaertidsskrift 67,pp. 805-811.

PETTIPHER,G.L., MANSELL,R., KINNON,C.H. and COUSINS,C.M./1980/: Rapid membrane filtration-epifluorescent microscopy technique for direct enumeration of bacteria in raw milk. Appl. Environm. Microbiol. 39, pp.423-429.

PETTIPHER,G.L. and RODRIGUES,U.M./1982/: Rapid enumeration of microorganisms in food by direct epifluorescent filter technique. Apol. Environm. Microbiol. 44,po.809-813.

RODRIQUES,U.M. and KROLL,R.G. /1986/: The Direct Epifluorescent Filter Technique for the enumeration of yeasts. J.Appl. Bacteriol. 61, po. 139-144.

RODRIQUES,U.M. and KROLL,R.G. /1988/: Rapid selective enumeration of bacteria in foods using a microcolony epifluorescence microscopy technique. Apol. Bacteriol. 64, po.65-78.

Fig.1 Total count relation between SPC and DEFT
- raw ground meat .——— .
- cured meat Δ— — —Δ

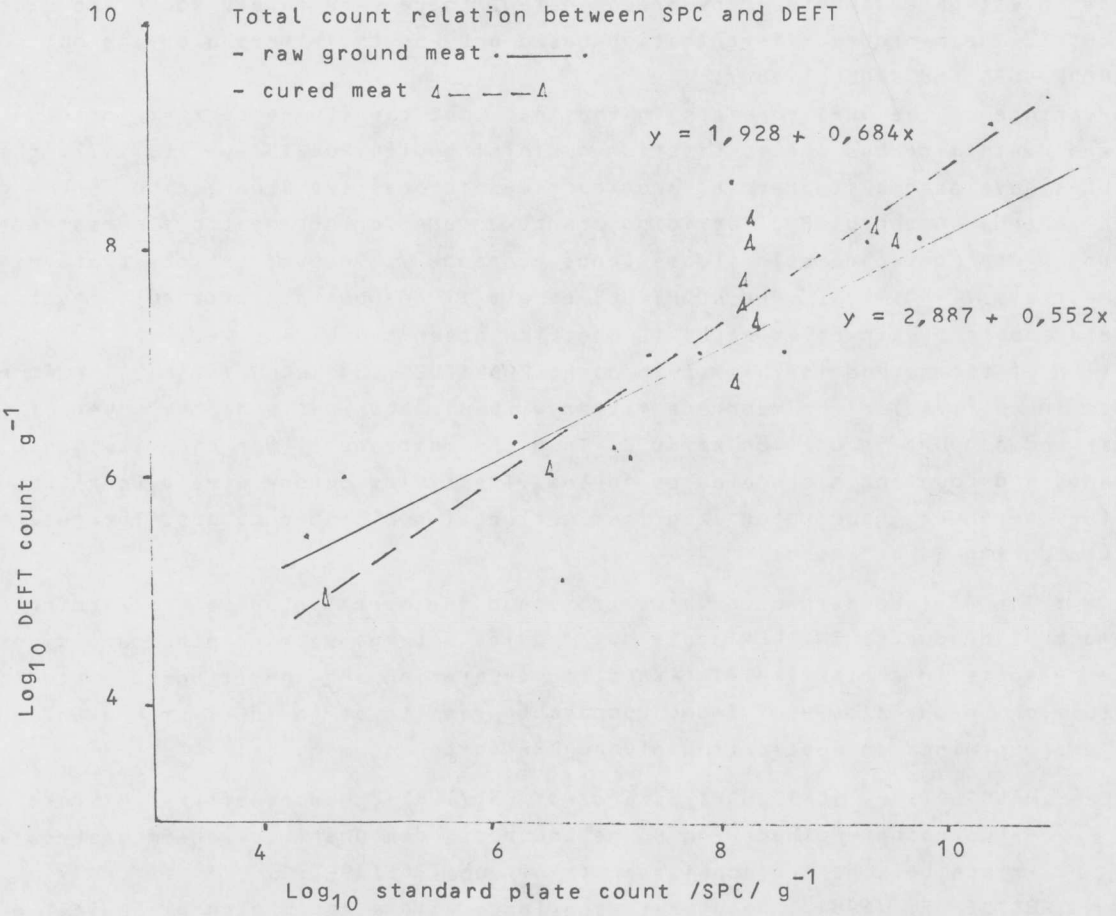

Fig.2

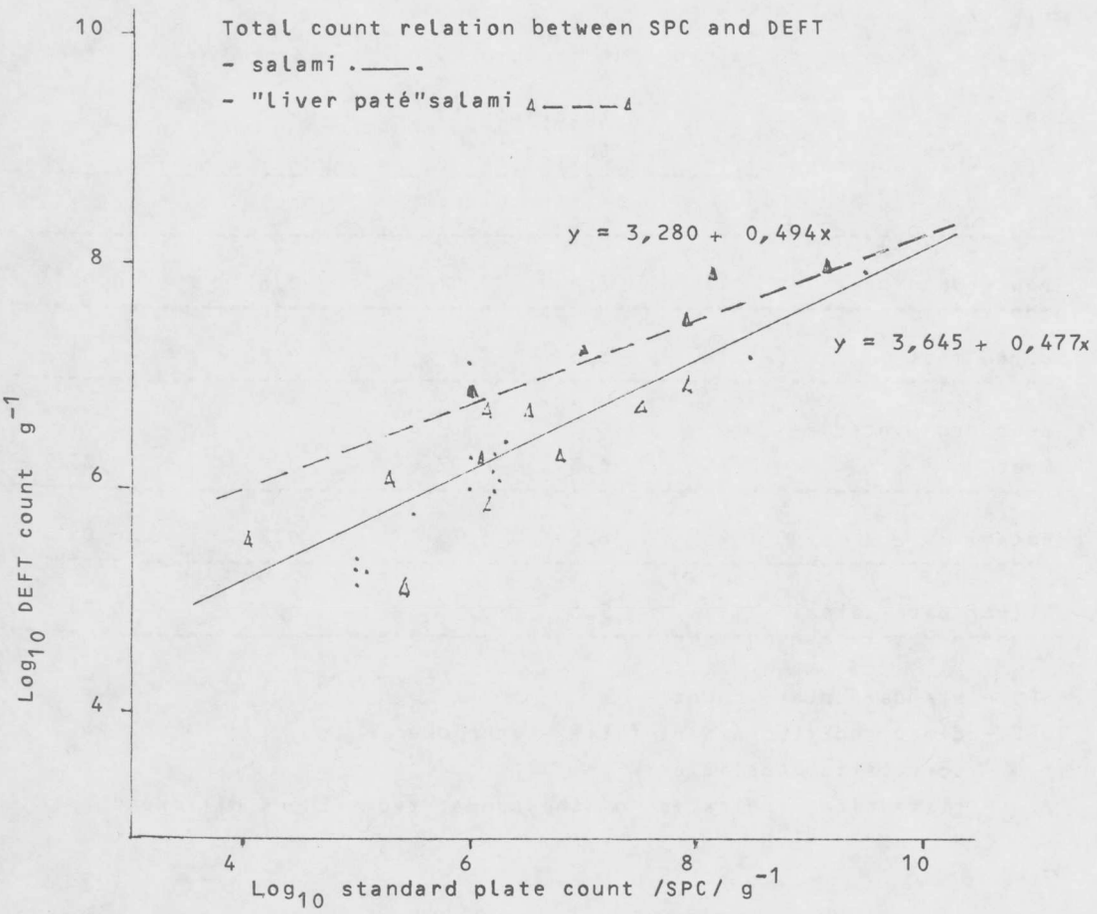
Total count relation between SPC and DEFT
- salami .——.
- "liver paté"salami Δ— — —Δ
y = 3,280 + 0,494x
y = 3,645 + 0,477x
Log10 DEFT count g-1
Log10 standard plate count /SPC/ g-1
10
8
6
4
4
6
8
10

Fig.3

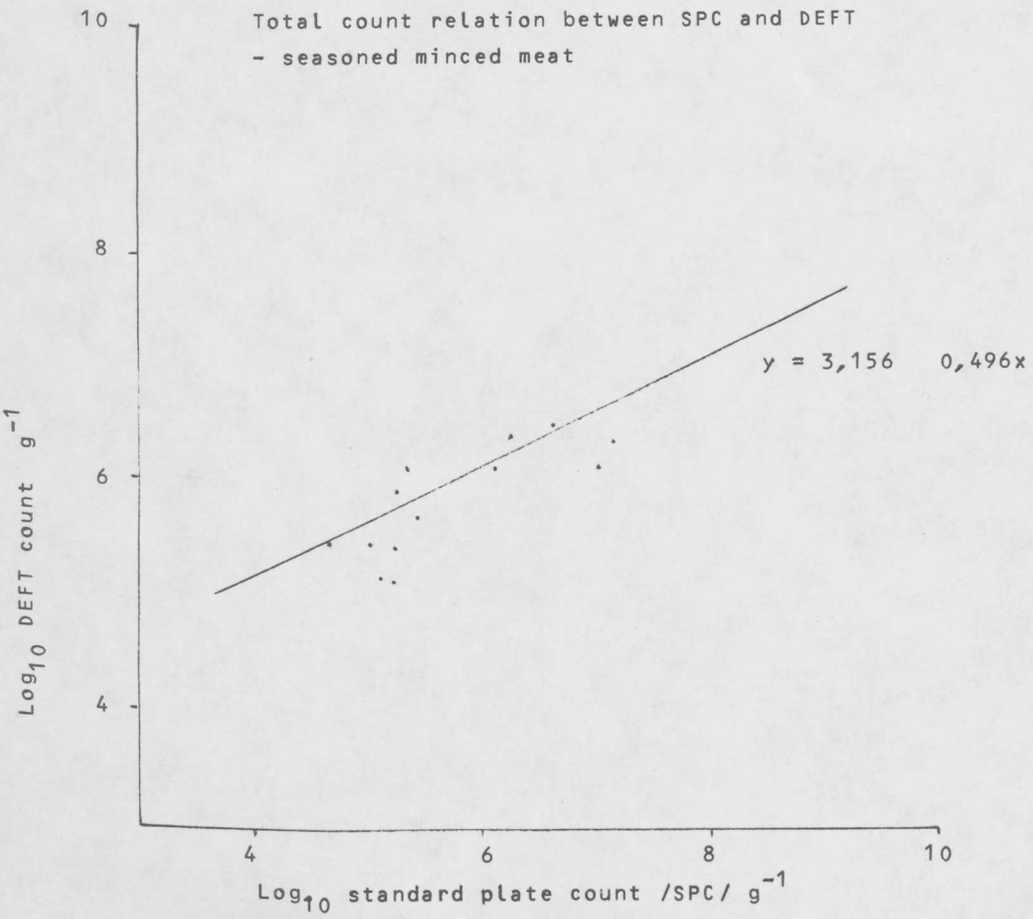
Total count relation between SPC and DEFT
- seasoned minced meat
y = 3,156 0,496x
Log10 DEFT count g-1
Log10 standard plate count /SPC/ g-1
10
8
6
4
4
6
8
10

Fig. 4

Sampl	n	total count $\log_{10} g^{-1}$		r	p
		SPC	DEFT		
raw ground meat	15	7,4	7,01	0,87	>0,05
cured meat	10	8,5	7,7	0,82	<0,01
seasoned minced meat	15	6,3	6,27	0,86	>0,05
salami	15	6,5	6,7	0,81	>0,05
"liver paté" salami	15	5,8	6,1	0,79	>0,05

SPC - standard plate count
DEFT- direct epifluorescent filter technique
r - correlation coeficient
p - statistical indication of the comparated methods difference / T-test/