***Standard Plate Count (SPC)***

 **This method consists of growing the bacteria in a nutrient culture petridish or (petrifilm) and counting colonies which develop. It can be used for all types of dairy products and is generally used in the examination of Grade A raw and pasteurized milk. This method used to determine the general quality of the milk supply.**

***Procedure***

1. **Wash the hands and disinfect the stage.**
2. **Mixing of milk sample.**
3. **Make a series dilutions of milk sample.**
4. **Transfer a suitable dilution to a petridish by sterile pipette.**



 **5. Add a suitable amount (12 ml ) from SPC agar on the dilution and mixing**

 **gently by moving the dish on different direction.**

 **6. Incubate 48 hours at 32°C .**

 **7. Count colonies and report results.**

**Note: Choosing the plates with ( 25 - 250 ) colonies**

***SPC(Cfu/ml)= Average number of colonies x Reciprocal of the dilution used***

***Counting: mean estimate of bacterial populations and it is not exact because:-***

1. **The agar used in method (SPC agar) is not suitable for growing all species of bacteria.**
2. **Incubation Degree which used in method (32°C) is not suitable for growing all species of bacteria.**
3. **Sharing more than one bacteria to form one colony.**

***Duplicate plate dilution***

***Note: SPC(Cfu/ml)= Average number of colonies x Reciprocal of the dilution used***

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|  **Only one dilution yields plates with 25-250 colonies compute the mean for that dilution as the basis for the SPC .**  | **1/1000** | **1/100** |
| **16** | **175** |
| **17** | **208** |
| **175+208=383 383÷2=191.5 191.5x100=19150….SPC=19000**  |

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| **1/100** | **1/1000** |  **Both dilutions yield plates with 25 to 250 colonies: Average the mean count for each dilution as the basis for SPC.** |
| **230** | **28** |
| **246** | **36** |
| **230+246=476****476÷2=238****238x100=23800**…**SPC=23800** | **28+36=64****64÷2=32****32x1000=32000 SPC=32000** |
| **23800+32000=55800÷2=27900… SPC= 28000**  |

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| ***unless* the count computed for the higher dilution is more than twice the computed for the lower dilution .In the latter instance , use the lower computed count as the SPC.**  | **1/1000** | **1/100** |
| **42** | **138** |
| **30** | **162** |
| **42+30=72****72÷2=36****36x1000=36000…SPC=36000** | **138+162=300****300÷2=150****150x100=15000… SPC=15000** |

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| **Neither dilution yields plates with 25-250 colonies: Use the mean count the lower dilution as the basis for estimating the SPC.**  | **1/1000** | **1/100** |
| **23** | **287** |
| **19** | **263** |
|  | **287+263=550****550÷2=275****275x100=27500… ESPC=28000** |

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| **Both dilutions yield only plates with fewer than 25 colonies: Use the mean count of the duplicates of the lowest dilution to** **estimate the Spc** | **1/1000** | **1/100** |
| **2** | **18** |
| **0** | **14** |
|  | **18+14=32****32÷2=16****16x100=1600**…….**ESPC=1600** |

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| **Both dilutions yield plates with no colonies: Estimate the SPC as less than (<) 1 times the lowest dilution .**  | **1/1000** | **1/100** |
| **0** | **0** |
| **0** | **0** |
|  | **Lower than 1x100****ESPC=lower than 100** |

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|  **Only one plate of one dilution contains 25-250 colonies: When one plate of one dilution contains 25-250 colonies and the duplicate contains more than 250 colonies , use both plates in computing the SPC.**  | **1/1000** | **1/100** |
| **23** | **272** |
| **19** | **248** |
|  | **272+248=520** **520÷2=260****260x100=26000**………**SPC=26000** |

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| **One plate of each dilution contains 25-250 colonies and the duplicate contains more than 250 or less than 25 colonies :use all four in computing the SPC.**  | **1/1000** | **1/100** |
| **22** | **275** |
| **35** | **240** |
| **22+35=57****57÷2=28.5****28.5x1000=28500**…**SPC=29000** | **275+240=515****515÷2=257.5****257.5x100=25750**…..**SPC=26000** |
| **26000+29000 = 55000 ÷2 = 27.500 …SPC=28000** |

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| **Both plates ;one of them(lower dilution) TNTC ,the other (high dilution )with 10 - 100 colonies per cm² : use the estimated count of the ESPC.** **In this case take the average of (4 square cm²).** | **1/1000**  | **1/1oo**  |
| **14,20,34,12 TNTC**  | **TNTC**  |
| **SPC(Cfu)=Average of(4 square cm)x 65 cm²xreciprocal of dilution**  **= 20 x 65 x 1000**  |

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| **Both plates ;one of them(lower dilution) TNTC ,the other (high dilution )with less than 10 colonies per cm²: use the estimated count of the ESPC. In this case take the average of(12 square cm²).** | **1/1000**  | **1/100**  |
| **3,6,7,8,2,5,3,2,7,9,6,3**  | **TNTC**  |
| **SPC(Cfu)=Average of(4 square cm)x 65 cm² x reciprocal of dilution = 5 x 65 x 1000**  |