Bioma, Desember 2023
Vol. 25, No. 2, Hal. 88-91

p ISSN: 1410-8801
e ISSN: 2598-2370

Total Plate Number Test at 0.5 McFarland Standard in *Escherichia coli* Culture

Tirta Setya Bhakti[1*], Ratna Porwanti[1], Dewi Rahmawati[2] dan Putik Pribadi[1]
[1]National Agency of Drug and Food Control in Semarang, Semarang
[2]National Agency of Drug and Food Control in Banjarmasin, Banjarmasin
Corresponding author : tirta.bhakti@pom.go.id

Abstract

Estimation of the number of bacteria can be calculated quickly by comparing the turbidity of the test bacterial suspension against the McFarland standard. In the microbiology laboratory, McFarland standard of 0,5 is often used as reference. There are still few scientific references about the estimated enumeration of bacteria according to McFarland standards. The purpose of this study was to provide basic information on the estimated number of *E.coli* bacteria according to the McFarland standard of 0,5. The analytical method uses ISO SNI 16649-2: 2001 for the enumeration *E.coli* using TBX Agar Media with 10 replications to get a good average result. The result of *E.coli* enumeration with the 0,5 McFarland standard was 4,0 x 10^7 colonies/mL. Thus the approximate information on the bacteria enumeration could be done quickly.

Keywords: *E.coli, McFarland 0,5 standard, TBX Agar Media*

Abstrak

Perkiraan perhitungan jumlah bakteri secara cepat dapat dilakukan dengan membandingkan kekeruhan suspensi bakteri uji terhadap standar McFarland. Pada laboratorium mikrobiologi standar McFarland 0,5 merupakan standar yang sering digunakan. Masih sedikit referensi ilmiah tentang perkiraan perhitungan jumlah bakteri yang sesuai standar McFarland. Tujuan penelitian adalah memberikan informasi dasar perkiraan jumlah bakteri *E.coli* sesuai standar McFarland 0,5. Metode analisa menggunakan acuan ISO SNI 16649-2: 2001 tentang uji angka lempeng total *E.coli* menggunakan TBX Agar Media dengan 10 kali ulangan untuk mendapatkan hasil rata-rata yang baik. Hasil perhitungan uji angka *E.coli* dengan standar McFarland 0,5 adalah 4,0 x 10^7 koloni/ mL. Dengan demikian informasi perkiraan perhitungan jumlah bakteri dapat dilakukan dengan cepat.

Kata kunci: *E.coli, standar McFarland 0,5, TBX Agar Media*

INTRODUCTION

Calculation of the number of bacteria can be done directly and indirectly. Direct calculations are used to count the total number of both dead and live bacteria, while indirect calculations are used to determine only live bacteria (Rosmania et al. 2020). Calculation of bacteria by turbidimetry (turbidity) is an example of an indirect calculation using a spectrophotometer.

In microbiology laboratories, the McFarland standard is a standard used as a reference for adjusting the turbidity of bacterial suspensions or standardizing testing microbes so that the number of bacteria is within the given range (Fitri et al, 2015). McFarland's standard is made from a solution of barium chloride and sulfuric acid, which react to produce a fine precipitate of barium sulfate. Before use, shake well to ensure the barium sulfate is evenly distributed throughout the solution. This standard is sensitive to air and light, make sure the tube is tightly closed at all times and store it in a dark place. McFarland standards should be checked periodically to ensure no evaporation is taking place. Discard if there is a reduction in volume. McFarland Standard should be shaken or vortexed each time it is used and checked for turbidity. McFarland Standard should be discarded if any particles appear or agglomeration occurs. There are

various types of McFarland standards, namely the McFarland standard starting from 0,5 to the McFarland Standard 10.

Arimbi (2017) states that the Mcfarland standard most commonly used in the Clinical Microbiology Laboratory is the Mcfarland 0,5 standard, which is the basis for conducting antimicrobial susceptibility experiments and testing bacterial culture results. There is little official information or research regarding estimates of bacterial counts, by the McFarland standard. The purpose of this study was to provide information to determine the approximate number of *E.coli* bacteria that grow equivalent to the McFarland standard of 0,5 so that the number of bacteria can be calculated more quickly and also can be used as information in evaluating the amount of bacterial inoculum. This research uses validated methods, and uses more specific reagents or media so that it can provide more relevant results.

METHODS

The test bacteria used pure lyophilisate standard for E.coli bacteria obtained from P30MN, BPOM. Lyophilisate was taken as much as 30 µL using a sterile loop, put into 10 mL of BHIB solution, incubated for 24 hours at 35-37 ºC. Then it was streaked onto 1 TSA plate, incubated 24 hours at 35-37 ºC. Cultures growing on TSA were harvested by adding 10 mL of 0,85% NaCl, then the cultures were taken using sterile loop. The culture mixture and 0,85% NaCl are collected into a sterile tube. Then, 20 µL were taken from the culture mixture using sterile loops, put into a sterile tube containing 3 mL of 0,45% NaCl and measured by the turbidity method using the Densicheck Plus from Biomerieux. Measurement of the concentration of the solution with Densicheck Plus Biomerieux was carried out after Densicheck was verified using 3 standard solutions: 0,0 McFarland, 0,5 McFarland, 2,0 McFarland and 3,0 McFarland. Bacterial suspension readings were carried out with 3 repetitions to obtain good homogeneity. The test bacterial suspension was diluted up to 10 levels of dilution using a peptone-salt solution as a solvent.

The analytical method used in this study refers to ISO SNI 16649-2: 2001 concerning the test for *E.coli* numbers in food using TBX Agar selective media. Calculate the number of bacterial colonies using the Total Plate Count method with the help of a Colony Counter. The bacterial suspension was tested by pouring method with 10 repetitions of the test to obtain homogeneous data.

RESULTS AND DISCUSSION

The results of the calculation of the test bacteria using the *E.coli* number test method obtained the results according to table 1 and table 2.

Table 1: The results of the Total Plate Count of *E. coli* replicates 1-5.

Dilution	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5
10^{-1}	> 300	> 300	> 300	> 300	> 300
10^{-2}	> 300	> 300	> 300	> 300	> 300
10^{-3}	> 300	> 300	> 300	> 300	> 300
10^{-4}	> 300	> 300	> 300	> 300	> 300
10^{-5}	> 300	> 300	> 300	> 300	> 300
10^{-6}	35	35	35	38	41
10^{-7}	10	12	12	13	14
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

Table 2: The results of the Total Plate Count of *E. coli* replicates 6-10.

Dilution	Repetition 6	Repetition 7	Repetition 8	Repetition 9	Repetition 10
10^{-1}	> 300	> 300	> 300	> 300	> 300
10^{-2}	> 300	> 300	> 300	> 300	> 300
10^{-3}	> 300	> 300	> 300	> 300	> 300
10^{-4}	> 300	> 300	> 300	> 300	> 300
10^{-5}	> 300	> 300	> 300	> 300	> 300
10^{-6}	41	43	44	45	45
10^{-7}	15	15	20	21	23
10^{-8}	0	0	0	0	0
10^{-9}	0	0	0	0	0
10^{-10}	0	0	0	0	0

The test bacterial suspension was made by culturing pure *E.coli* bacteria with an incubation period of 24 hours to obtain a good culture. Pure culture is a culture whose cells come from the division of a single cell. Colonies that grow white on TSA plate media. *Escherichia coli* is a gram-negative, bacillus-shaped bacterium, about 2 micrometers long and 0,5 micrometers in diameter.

From the measurement results for the preparation of the test suspension by the Densicheck tool with 3 replications, the turbidity measurement results for the *E.coli* test bacteria were 0,5, 0,5, and 0,5. Bacterial suspension testing using a quantitative method of *E.coli* according to ISO SNI 16649-2: 2001. Selective media using TBX (Tryptone Bile X-glucoronide) Agar Media.

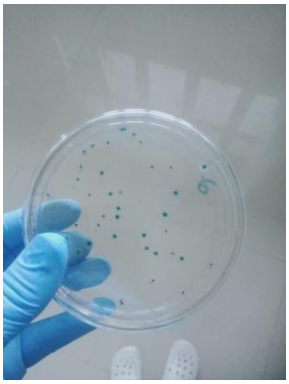

Figure 1. Growth of *E.coli* on TBX Agar Media

Figure 2. The measurement results of the test bacterial suspension were equivalent to 0,5 using the Densicheck tool.

From research at the BBPOM laboratory in Semarang, the results obtained for the number of *E.coli* with an average of 10 repetitions according to the turbidity equivalent to the McFarland standard 0,5 was 4,0 x 10^7 colonies/mL. A similar study was conducted by Rosmania (2020), the number of *E.coli* at turbidity equivalent to the McFarland standard 0,5 obtained results of 1,3 x 10^7 colonies/mL using Nutrient Agar Media. Differences in calculation results occur due to differences in media usage.

TBX Agar Media is more specific for the growth of *E.coli* bacteria and produces contrasting colonies that make calculations easier. This media is more specific for detecting or differentiating *E.coli* bacteria from other coliforms in the presence of a glucuronidase enzyme which is highly specific for *E.coli*. The content of X-glucoronide in this media helps detect the activity of the glucuronidase enzyme in *E. coli* bacteria. *E.coli* cells absorb X-glucoronide and then the glucuronidase enzyme separates the bond between the chromophore and the glucuronide. The released chromophore will give it a distinctive green-blue color. The bile salt mixture inhibits Gram-positive bacteria. Basically growth is the result of metabolism, a directed chemical reaction that takes place in cells catalyzed by enzymes (Subagiyo et al, 2015). In the field of microbiology, the development of culture media for bacteria is an important point because by isolating bacteria and growing them with artificial media, one can identify and study the properties of a bacterium. A microorganism requires nutrition as a condition for its growth (Candrasari et al., 2011).

Nutrient Agar Media is a medium that is commonly used to grow bacteria. Colonies that grow will be white. This colony is relatively almost the same as the color of the medium.

A mixture of physiological peptone and NaCl in the Peptone-Salt Solution content has a better nutritional content when compared to a solution containing only peptone. Media Peptones provide essential growth nutrients for organisms (Dede et al. 2014). This peptone is also the main nitrogen source in commercial media for microbial growth which consists of a mixture of polypeptides, dipeptides, and amino acids which can be obtained from protein-containing materials through acid or enzymatic hydrolysis reactions (Fachraniah et al. 2002) while istonic solutions contain Physiological NaCl is useful for the recovery of bacterial cells from vulnerable conditions to cells that are ready to grow.

The incubation period for testing the *E.coli* number is 18-24 hours. According to Dwidjoseputro (1994), incubation for 24 hours is possible because at that time the bacteria are already in the logarithmic or exponential phase. In this phase, the bacteria are constantly dividing and the number of cells increases. Thus, the metabolic activity of the bacterial culture is in an optimal phase so that research will be relevant if it is carried

out when the bacteria have optimal metabolic activity.

CONCLUSION

The results of testing the E.coli count equivalent to McFarland standard 0,5 according to ISO SNI 16649-2: 2001 using TBX Agar specific media is 4,0 x 107 colonies/mL. This provides basic information about the estimated bacterial counts so that calculations can be performed more quickly. Recommendations for further research are variations in the types of bacteria and test methods so that evaluation data for comparison of the number of bacteria on the MCFARLAND standard can be obtained.

ACKNOWLEDGMENT

Our thanks go to Ms. Dra Aryanti, M.Si Apt as Coordinator of BBPOM Testing in Semarang, Mrs. Sri Hartati, M.Sc, Apt as sub-coordinator of BBPOM Microbiology Testing in Semarang, Mr. Alfi Sophian M.Si from P30MN of Indonesian Agency of Drug and Food Control for grant permission to conduct research and assist in conducting briefings in discussing test results.

REFERENCES

Arimbi, A.S. 2017. Aktivitas Antibakteri Kombinasi Fraksi Daun Moringa oleifera dan Ekstrak Daun Persea americana (Studi Terhadap Bakteri Staphylococcus aureus dan Escherichia coli dengan Metode Difusi Cakram). Universitas Muhammadiyah Malang.

Candrasari A, Romas M.A, Hasbi M dan Astuti O.R. 2011. Uji Daya Antimikro Ekstrak Etanol Daun Sirih Merah (Piper crocatum Ruiz & Pav.) Terhadap Pertumbuhan Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 11229 dan Candida albicans ATCC 10231 Secara In Vitro. Biomedika 4 (1). 10.23917/biomedika. v4i1.258

Chambers, Henry F. 2004. The Changing Epidemiology of Staphylococcus aureus. In: CDC Past Issue. Vol 7 (2). 10.3201/eid0702.010204

Dwidjoseputro, D. 1990. Dasar-dasar Mikrobiologi.

Fachraniah, Fardiaz. D. dan Idiyanti. T. 2002. Pembuatan Pepton dari Bungkil Kedelai dan Khamir dengan Enzim Papain Untuk Media Pertumbuhan Bakteri. Jurnal Teknologi dan Industri Pangan 13 (3).

Fitri K, S.A., Agung, M.U.K., dan Meika J. 2015. Larutan McFarland Standar digunakan sebagai Referensi untuk menyesuaikan Kekeruhan Bakteri Suspensi Sehingga Jumlah Bakteri Dalam Kisaran yang diberikan Untuk Membakukan Mikroba Pengujian. Jurnal Akuatika 6 (A) 128-139.

Oktavia, N., dan Pujiyanto, S. 2018. Isolasi dan Uji Antagonisme Bakteri Endofit Tapak Dara (Catharanthus roseus, L.) Terhadap Bakteri Escherichia coli dan Staphylococcus aureus. Berkala Bioteknologi 1 (1) 1-7.

Rosmania, F.Y. 2020. Perhitungan Jumlah Bakteri di Laboratorium Mikrobiologi menggunakan Pengembangan Metode Spektrofotometri. Jurnal Penelitian Sains 22 (2) 76-86.

Saputra, D dan Tati. N. 2013. Produksi dan Aplikasi Pepton Ikan Selar Untuk Media Pertumbuhan Bakteri. JPHPI Volume 16 Nomor 3.

Subagiyo, S. M. dan Triyanto W. A, S. 2015. Pengaruh pH, Suhu dan Salinitas Terhadap Pertumbuhan dan Produksi Asam Organik Bakteri Asam Laktat yang Diisolasi Dari Intestinum Udang Penaeid. Jurnal Ilmu KelautanVol 20 (4) 233-240.