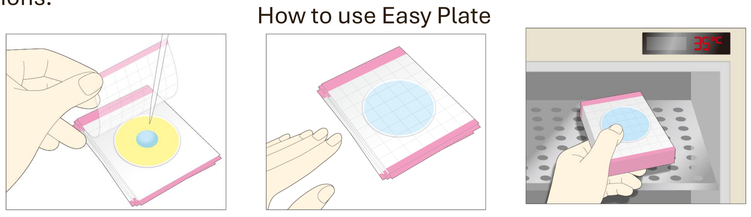
Evaluation of Accuracy and Efficiency for Novel Automatic Colony Counting System for Ready-to-Use Culture Media, Easy Plate™

Shinichiro Sugiura[1], Kentaro Takenaka[2]
(1) Kikkoman Biochemifa Company, (2) Kikkoman Corporation

Introduction

Ready-to-use (RTU) culture media enhances food safety and productivity because of its quickness, compactness, simplicity, and visibility of colonies compared to conventional agar plates. **Easy Plate AC for aerobic bacteria count** and **Easy Plate CC for coliform bacteria count** are AOAC PTM certified[1, 2] and MicroVal certified[3] RTU dried medium that spreads sample suspension evenly over the plate surface by simply closing the cover film. It also has advantages over conventional agar plates, such as reduced plastic usage and reduced GHG emissions.

How to use Easy Plate



Colonies on Easy Plate are easy to count because they form bright, high-contrast colonies, but manual counting is time-consuming and causes artificial errors.

Colony counting system for Easy Plate (CCS), jointly developed by Kikkoman Biochemifa Company and NTT DATA BUSINESS SYSTEMS Corporation, is an automated counting software dedicated for Easy Plate and has the following features.

- **AI-based** image recognition algorithm
- **No need to adjust parameters** according to the sample
- CCS only needs **a general-purpose document scanner**, thus reducing the initial investment
- Simple and **easy-to-use UI**
- All 6 types of Easy Plates supported

System configuration of CCS

Category	Detail
PC	OptiPlex 3080 SFF(Dell), Windows10, Corei3-10105(4C/3.7-4.4GHz) /16GB/256GBSSD+1TBHDD
Scanner	ADS-4300N (Brother Industry, LTD)
Scanner software	Brother ScanEssentials Ver. 1.1.0.2(Brother Industry, LTD)
Counting software	CCS for Easy Plate Ver. 1.0.2*

*The latest version is Ver. 1.2.1

Results - Efficiency

Conventional manual counting took time in proportion to the number of colonies. On the other hand, the CCS method showed an average time of 5.8 seconds/plate, regardless of the contamination level or the type of Easy Plate (Figure 1).

Compared to manual counting, the CCS method was 3.3 times faster for low contamination levels (10-50 CFU/plate) and 11.1 times faster for high contamination levels (150-300 CFU/plate) (Figure 2).

Since the CCS method automatically counts all images at once after scanning, it was found that the more plates processed in a batch, the more efficient the method could be, as the counting time per plate was reduced (Figure 3).

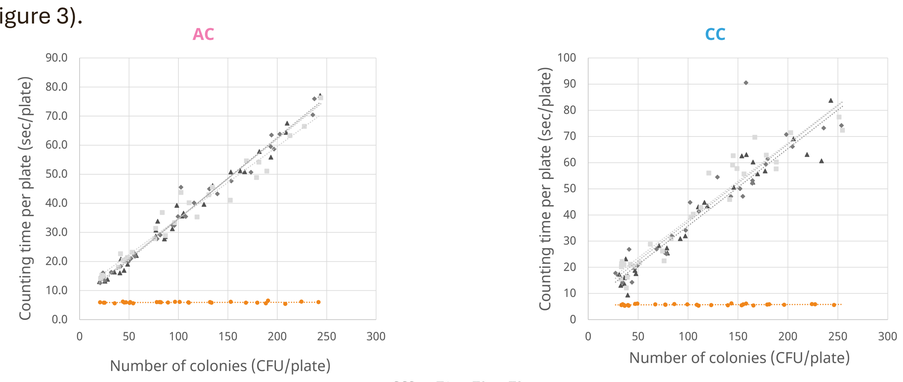


Figure 1. Comparison of counting speeds between counting methods

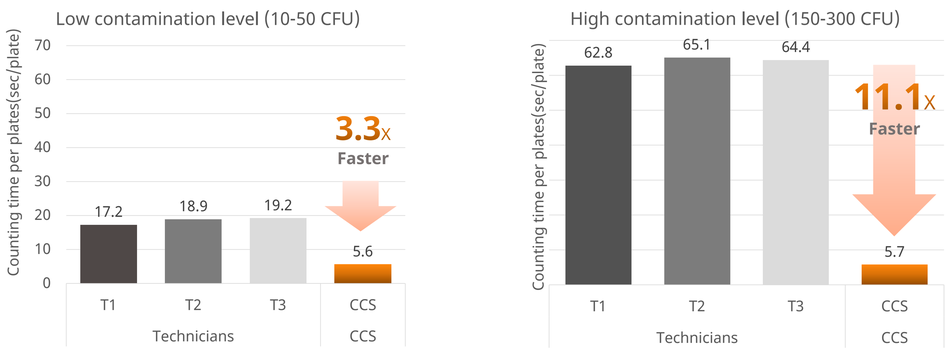
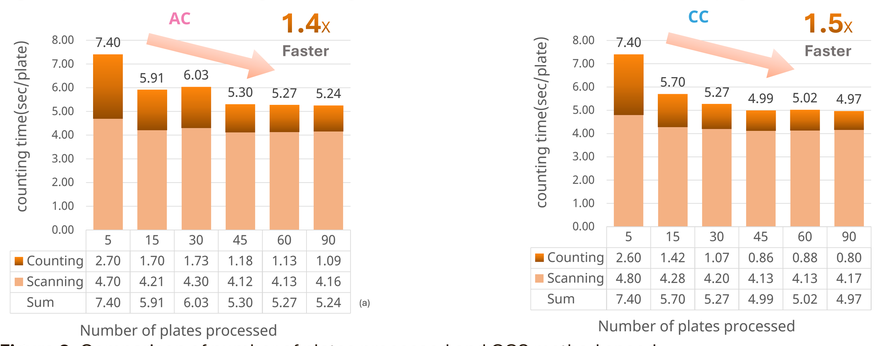


Figure 2. Comparison of average counting speeds between methods at (a)low and (b)high contamination



	5	15	30	45	60	90
Counting	2.70	1.70	1.73	1.18	1.13	1.09
Scanning	4.70	4.21	4.30	4.12	4.13	4.16
Sum	7.40	5.91	6.03	5.30	5.27	5.24

	5	15	30	45	60	90
Counting	2.60	1.42	1.07	0.86	0.88	0.80
Scanning	4.80	4.28	4.20	4.13	4.13	4.17
Sum	7.40	5.70	5.27	4.99	5.02	4.97

Figure 3. Comparison of number of plates processed and CCS method speed

Objectives

In this study, we evaluated the accuracy of conventional manual counting and automatic counting with CCS **(CCS method)** for **Easy Plate AC (AC)** and **Easy Plate CC (CC)** and compared their efficiency.

CCS method workflow

Materials and Methods

Sample preparation

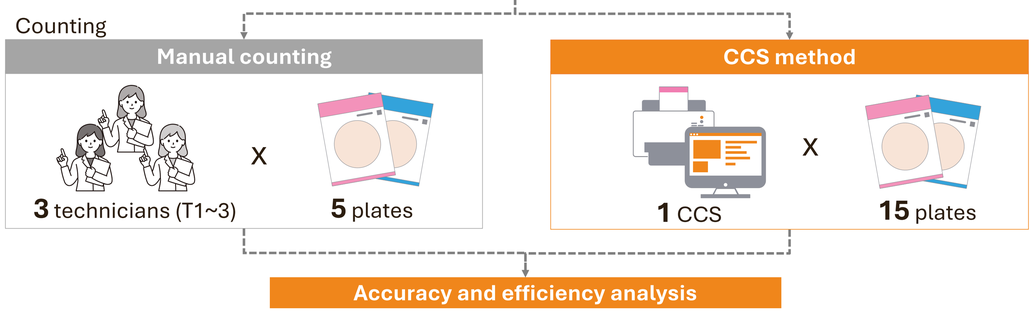
10 food samples	Contamination	pH adjustment
Raw ground beef	Naturally contaminated (35℃ 0-5 hours)	No adjustment
Raw ground chicken		
Onion salad		
Bean sprout		
Raw shrimp		
Tortilla roll		
Raw salmon	*Escherichia coli* NBRC 15034 was inoculated	
Frozen pizza		
Kikkoman soymilk		
Vegetable juice		pH 7 using 1N NaOH

Stomached and diluted in phosphate-buffered saline (PBS)

Inoculation & Incubation

	AC	CC
Medium, type of Easy Plate	For aerobic count	For coliform count
Incubation temperature & time	35±1℃, 48±2hr	35±1℃, 24±1hr
Plates	15 plates	
Contamination level(CFU/plate)	Low: 10-50, Middle: 50-150, High: 150-300	

Counting



Results - Accuracy

The count results of CCS method both AC and

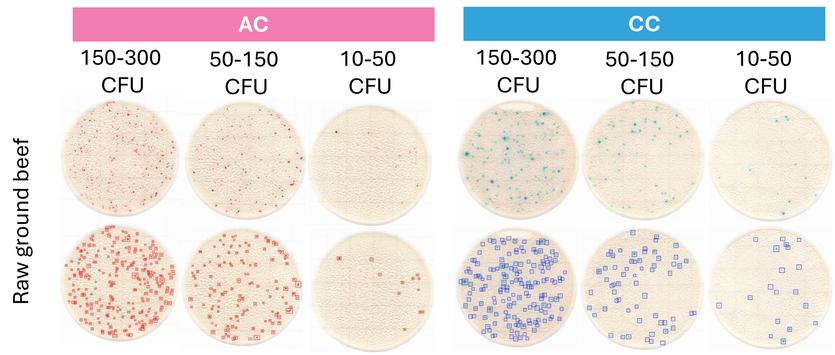


Figure 4. Marked and not marked AC and CC plate image

Correlation coefficients between manual counting and the CCS method were greater than 0.98 for both AC and CC (Figures 5a and 5b). However, in some cases, such as in areas X and Y, no correlation was obtained.

It is assumed that the differences in area X due to the false detection of bubbles in CCS method (blue arrow in Figure 5c) and the missing of small colonies in manual counting (orange arrow).

The differences in area Y may be due to the presence of very thin colonies that did not show up on the scanner in CCS method (blue arrows in Figure 5d) .

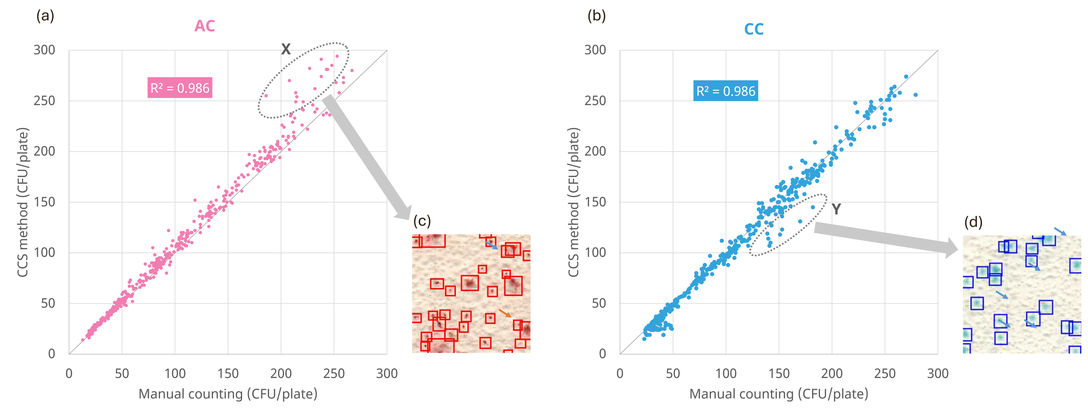


Figure 5. Correlation coefficients of (a) AC and (b) CC for bacterial counts between counting methods. (c) Portion of AC in area X and (d) CC in area Y.

Conclusions

- The counting speed is **faster** when more plates are processed at a batch.
- The CCS method uses a general-purpose scanner, which makes it **a low-cost option** for users.
- The CCS method is an effective way to **reduce counting time while maintaining accuracy.**

References

1. OKOCHI, Norihiko, et al. Dai Nippon Printing Co., Ltd, Medi· Ca AC for Enumeration of Aerobic Bacteria. *Journal of AOAC International*, 2014, 97.3: 837-842.
2. SAITO, Fumihiko, et al. Dai Nippon Printing Co., Ltd Medi· Ca CC for Enumeration of Coliform Bacteria. *Journal of AOAC International*, 2015, 98.1: 62-70.
3. https://microval.org/en/issued-certificates/

Contact information

Shinichiro Sugiura
shsugiura@mail.kikkoman.co.jp
Kikkoman Biochemifa Company
https://biochemifa.kikkoman.com/e/kit/

LinkedIn

Facebook

Link to CCS info

SCAN ME