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Enumerating Chromogenic Agar Plates Using the Color QCOUNT® Automated Colony Counter

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Abstract

Over the past decade, chromogenic substrates have been used in culture media for the detection, isolation, identification, and enumeration of microorganisms. Agar plates with chromogenic substrates will produce colonies with various colors, and the hand-counting method used to differentiate and enumerate these colonies is time-consuming and laborious. The Color QCOUNT was developed to improve efficiency in the microbiology laboratory by automatically counting colonies by color. In this study, color colony counts prepared using mixed microbe cultures were compared using both automated and manual count methods. Inoculum levels were prepared by serial dilution in a phosphate buffer to span the total counting range of 30-300 CFU/mL, 26 chromogenic agar types were used, and a total of 581 plates were analyzed. Plates were prepared, inoculated, and incubated according to manufacturer's instructions, manually counted once by two scientists, and counted in duplicate automatically. The Pearson correlation coefficient comparing the automated and manual counts for the entire pooled population of data was 0.987. The slope and intercept for the linear regression line were 1.0067 and 0.031, respectively. The mean log value difference between the automated and manual count methods for pooled data was -0.042. The mean log value differences between the manual and automated counts demonstrated that 83.8% of the plates analyzed were within 0.1 log, and 98.2% were within 0.2 log. These results demonstrate that the Color QCOUNT automated colony counter is a suitable alternative to the standard method of manually counting colonies by color on chromogenic agar plates.

Introduction

Chromogenic culture media is becoming widely used for the rapid detection, identification and enumeration of microorganisms in food, water, and clinical samples. Chromogenic media incorporates enzymatic methods using specific chromogenic substrates added to primary selective and nonselective media. Colorless substrates produce characteristic colors on agar plates when cleaved by organism-specific enzymes. Manually counting colored colonies on chromogenic media is tedious and time-consuming. The Color QCOUNT, an automated colony counter developed by Spiral Biotech, Inc., is designed to save valuable time and improve efficiency in the microbiology laboratory.

The Color QCOUNT system automatically counts up to 6 colors in less than 1 second with a single button, using preset factory or user-defined color settings. Users define specific colors using a color spectrum (Figure 1), and the counted colonies are tagged with their respective colors (Figure 2). The Color QCOUNT has a flexible data management system that saves the counted plate images with the count data in a database, and may be recalled from the archive to review, edit, or print. It is designed for use with commercially available and laboratory-prepared agar plates.

Automated counting systems are generally acceptable if the automated counts are within 0.5 log10 of the manual count (1,3). This study was conducted to evaluate the performance of the Color QCOUNT automated colony counter versus the manual hand-count method of counting colonies by color on chromogenic agar plates.

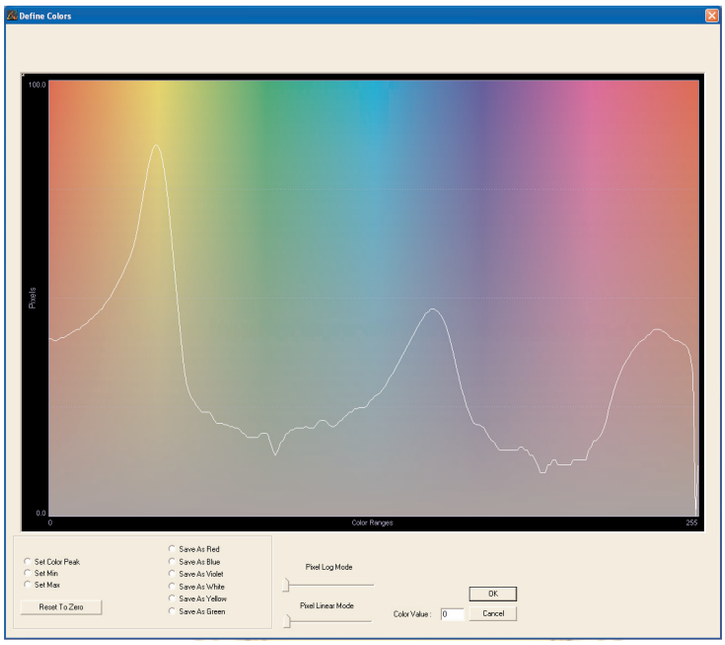

Figure 1: QCOUNT image used to define color ranges

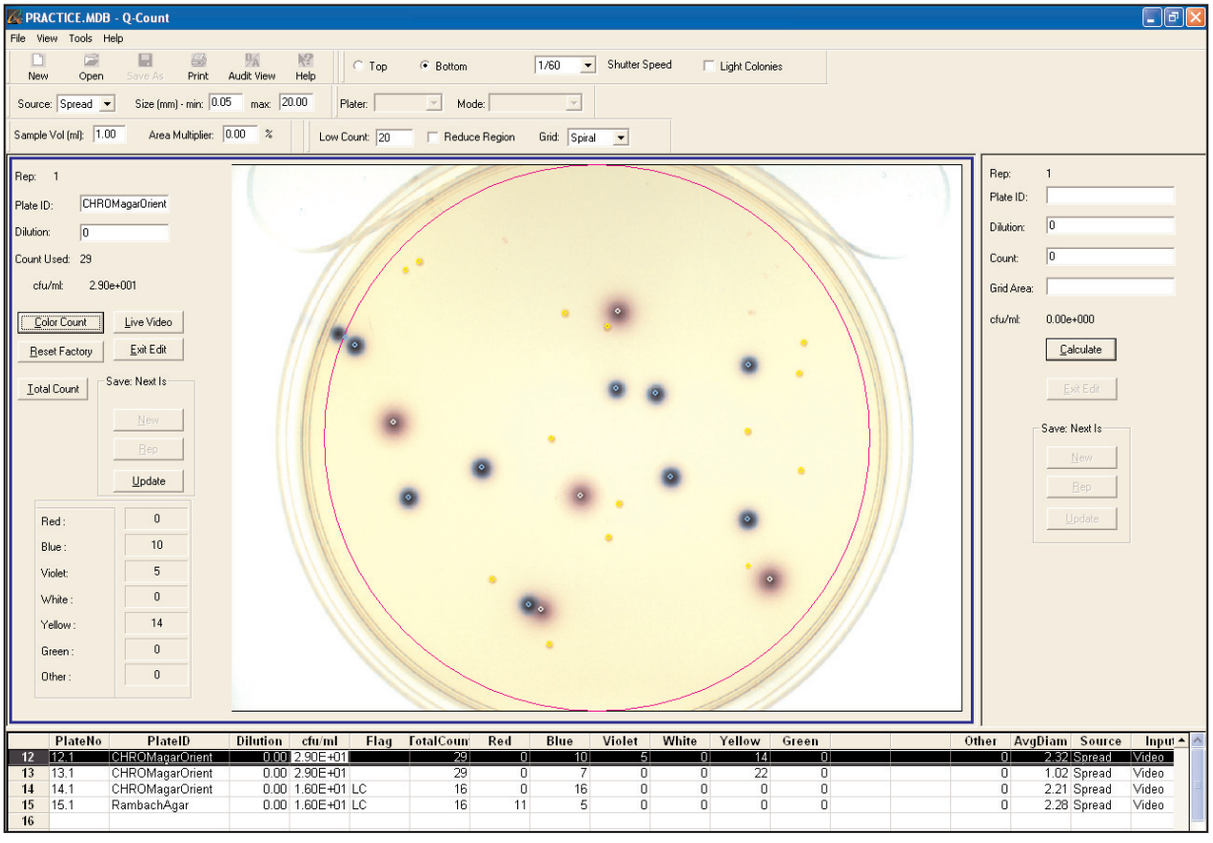

Figure 2: Counted color plate

Materials and Methods

Media and Microbe Preparation

Twenty-six chromogenic agar types and nine microbes were used in different combinations (Table 1). Inoculum levels adjusted to 0.5 McFarland turbidity were prepared by serial dilution in Butterfield's phosphate buffer to span the counting range of 30-300 CFU/mL. Plates were prepared, inoculated, and incubated according to each manufacturer's instructions.

Data Handling Method

Plates were manually counted by 2 scientists, and counted twice on the automated Color QCOUNT system (Spiral Biotech, Inc., Norwood, MA) with user-defined color settings. A 77mm circular template was used when counting plates manually, to match the automated colony counter's fixed counting region. The total counts for each type were averaged, and the total count for each plate/color was calculated. Any plate with a total count less than 30 or greater than 300 was excluded from further analysis. The base 10 logarithms of these colony counts were used for statistical analysis under the assumption that the transformed numbers would be normally distributed. A total of 581 plates were analyzed. Linear regression analysis for pooled data and the log differences for plate/color combinations between the manual and automated counts were determined using Microsoft® Excel 2000 (Microsoft Corporation).

Agar Types	Manufacturer[a]	Color QCount	Organism[b]
Rapid L'MONO agar	Bio-Rad Laboratories	Green Green	*Listeria monocytogenes* ATCC 7644 *Listeria ivanovii* ATCC 19119
CHROMagar™ ECC	CHROMagar Company	Blue Violet	*Escherichia coli* ATCC 25922 *Klebsiella oxytoca* ATCC 8724
CHROMagar™ Ecoli	CHROMagar Company	Blue Yellow	*Escherichia coli* ATCC 25922 *Salmonella typhimurium* ATCC 13311
CHROMagar™ Listeria	CHROMagar Company	Green Green	*Listeria monocytogenes* ATCC 7644 *Listeria ivanovii* ATCC 19119
CHROMagar™ Orientation	CHROMagar Company	Blue Violet Yellow Green	*Klebsiella oxytoca* ATCC 8724 *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 29213 *Enterococcus faecalis* ATCC 29212
CHROMagar™ Staph aureus	CHROMagar Company	Red Blue	*Staphylococcus aureus* ATCC 29213 *Enterococcus faecalis* ATCC 29212
CHROMagar™ Salmonella	CHROMagar Company	Red Blue	*Salmonella typhimurium* ATCC 13311 *Escherichia coli* ATCC 25922
Rambach Salmonella	CHROMagar Company	Red Blue	*Salmonella typhimurium* ATCC 13311 *Escherichia coli* ATCC 25922
Chromocult® Coliform	EMD Chemicals	Blue Violet	*Klebsiella oxytoca* ATCC 8724 *Escherichia coli* ATCC 25922
Chromocult® Coliform ES	EMD Chemicals	Blue Violet	*Klebsiella oxytoca* ATCC 8724 *Escherichia coli* ATCC 25922
HiChrome™ Coliform	HiMedia Laboratories	Blue Yellow Red/Violet	*Escherichia coli* ATCC 25922 *Klebsiella oxytoca* ATCC 8724 *Enterococcus cloacae* ATCC 13047
HiChrome™ ECC	HiMedia Laboratories	Blue Violet	*Escherichia coli* ATCC 25922 *Klebsiella oxytoca* ATCC 8724
HiChrome™ Ecoli	HiMedia Laboratories	Blue Yellow	*Escherichia coli* ATCC 25922 *Salmonella typhimurium* ATCC 13311
HiChrome™ Salmonella	HiMedia Laboratories	Red Blue	*Salmonella typhimurium* ATCC 13311 *Escherichia coli* ATCC 25922
HiChrome™ UTI	HiMedia Laboratories	Blue Blue Violet Yellow	*Klebsiella oxytoca* ATCC 8724 *Enterococcus faecalis* ATCC 29212 *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 29213
HiChrome™ Listeria	HiMedia Laboratories	Green Green	*Listeria monocytogenes* ATCC 7644 *Listeria ivanovii* ATCC 19119
RF® Listeria	R&F Products	Blue Blue	*Listeria monocytogenes* ATCC 7644 *Listeria ivanovii* ATCC 19119
RF® Sakazakii	R&F Products	Yellow Blue	*Escherichia coli* ATCC 25922 *Enterobacter sakazakii* ATCC 51329
RF® Salmonella	R&F Products	Red Blue	*Salmonella typhimurium* ATCC 13311 *Escherichia coli* ATCC 25922
BBL™ CHROMagar™ Staph aureus	BD Diagnostics	Red Blue	*Staphylococcus aureus ATCC 29213* *Enterococcus faecalis ATCC 29212*
BBL™ CHROMagar™ Salmonella	BD Diagnostics	Red Blue	*Salmonella typhimurium ATCC 13311* *Escherichia coli ATCC 25922*
BBL™ CHROMagar™ Listeria	BD Diagnostics	Green Green	*Listeria monocytogenes ATCC 7644* *Listeria ivanovii ATCC 19119*
BBL™ CHROMagar™ Orientation	BD Diagnostics	Blue Violet Yellow Green	*Klebsiella oxytoca ATCC 8724* *Escherichia coli ATCC 25922* *Staphylococcus aureus ATCC 29213* *Enterococcus faecalis ATCC 29212*
Oxoid Chromogenic Salmonella	Remel/Oxoid	Red Blue	*Salmonella typhimurium ATCC 13311* *Escherichia coli ATCC 25922*
Oxoid Chromogenic Listeria	Remel/Oxoid	Green Green	*Listeria monocytogenes ATCC 7644* *Listeria ivanovii ATCC 19119*
Oxoid Chromogenic Sakazakii	Remel/Oxoid	Yellow Blue	*Escherichia coli ATCC 25922* *Enterobacter sakazakii ATCC 51329*

a Bio-Rad Laboratories, Hercules, CA ; CHROMagar™ Company, Paris, France; EMD Chemicals through VWRI (only available in Canada); HiMedia® Laboratories Pvt. Limited, Mumbai, India; R&F® Products, Downer's Grove, IL; BD Diagnostics, Sparks, MD; Remel/Oxoid, Lenexa, KS.

b KWIK-STIK™ microorganisms were obtained from MicroBioLogics, St. Cloud, MN. ATCC® is a registered trademark of the American Type Culture Collection.

Table 1: List of chromogenic media and microbes used

Results and Discussion

Pooled Data Comparison

The results of the manual and automated colony counts were analyzed by linear regression, and the regression equation was y = 1.0067x + 0.031. The correlation coefficient for the regression line comparing the automated and manual count methods was 0.987 (Figure 3).

Figure 4 illustrates the performance limits of the automated versus manual counts for all chromogenic agar plate/color combinations. Of the 581 plates tested, 83.8% of the plates were within 0.1 log difference between the manual and automated counts, and 98.2% were within 0.2 log (Table 2).

The correlation coefficients for individual media and color combinations were all equal to or greater than 0.93 (Table 2), except for the CHROMagar Ecoli / yellow color, BBL CHROMagar Salmonella / blue color, and BBL CHROMagar Orientation / green color. The yellow color in CHROMagar Ecoli was difficult to count because the user had to reduce the light settings in order to suppress stray light near the edges of the plate. This change resulted in a darker image that did not show all yellow colonies on the plate. The BBL CHROMagar Salmonella blue colonies overlapped the red colonies such that the blue colonies were counted as red colonies. Green colonies on BBL CHROMagar Orientation were counted as blue because the green colonies overlapped the blue colonies, and green and blue wavelengths are adjacent to one another on the visible spectrum.

The mean log value difference between the automated and manual count methods for pooled data was -0.042. This small, negative dispersion is a result of: 1) poor contrast between background agar and colonies, and 2) plates with very complex morphologies, or overlapping colonies and colors. On chromogenic media, the colonies produce very dark colors in their centers, and this dark color tends to bleed toward the outer edges. When two colonies of different colors are very close together, it is difficult for both an automated counter and the human eye to discern color.

In this study, the Color QCOUNT selected colony colors based on user-defined color settings and counted the plates without editing for data analysis purposes. However, the QCOUNT has an editing feature where users can change the color of the colonies (Figure 5) and add/delete color colonies (Figure 6).

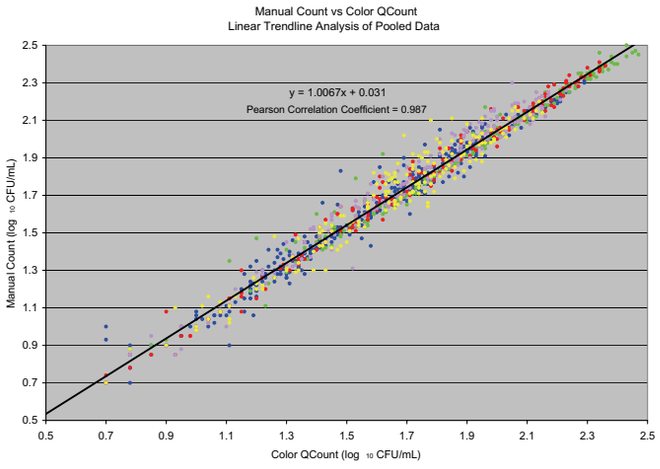

Figure 3: Linear trendline analysis of pooled data comparing automated and manual count methods

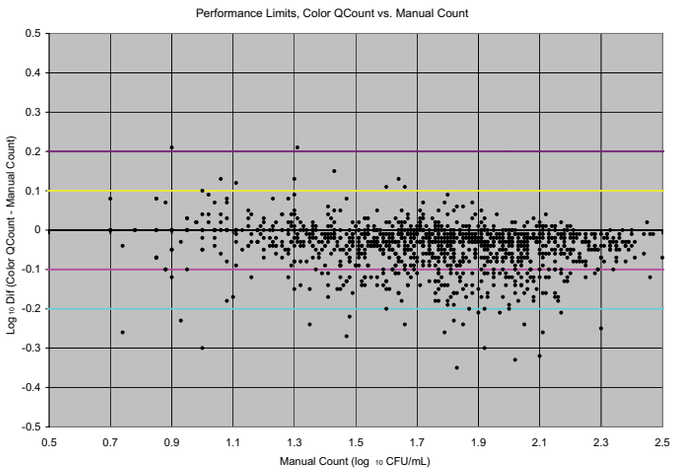

Figure 4: Performance limits demonstrating the mean log value differences between the automated and manual count methods

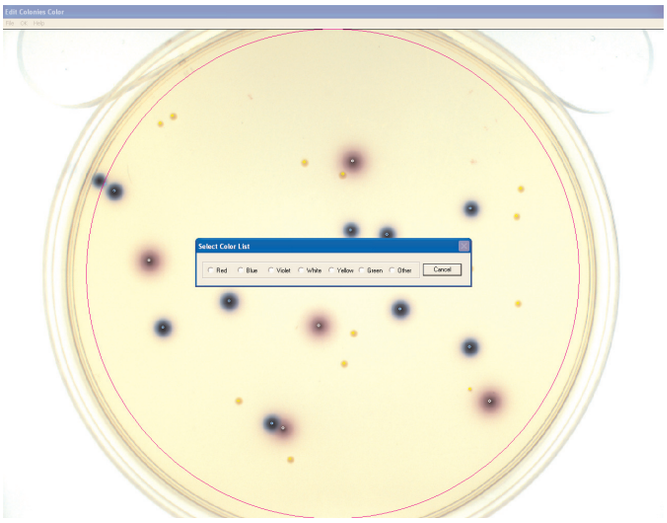

Figure 5: QCOUNT image of editing colony color function

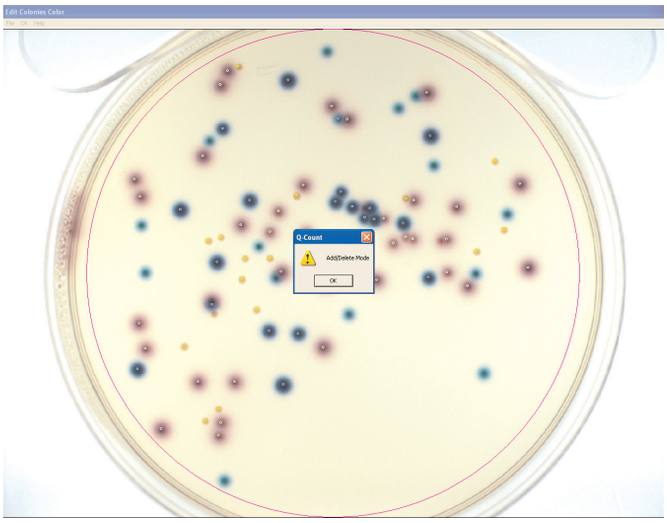

Figure 6: QCOUNT image of add/delete function

Agar types	No. of Plates	Color	Correlation Coefficient	Log_{10} Dif (Color Q - Manual) Distribution < -.2	< -.1	- .1 to .1	> .1	> .2
Rapid L'MONO agar	20	Green	0.93	2	6	14		
CHROMagar™ ECC	26	Blue	0.99		1	24	1	
		Violet	0.97			26		
CHROMagar™ Ecoli	26	Blue	0.96			26		
		Yellow	0.89	4	12	9	1	
CHROMagar™ Listeria	21	Green	1.00			21		
CHROMagar™ Orientation	21	Blue	0.99			21		
		Violet	0.99		3	18		
		Yellow	0.93		4	17		
		Green	0.96		1	20		
CHROMagar™ Staph aureus	22	Red	1.00			22		
		Blue	0.99			22		
CHROMagar™ Salmonella	23	Red	0.99		1	22		
		Blue	0.98		1	21	1	
Rambach Salmonella	22	Red	0.94		8	14		
		Blue	0.99			22		
Chromocult® Coliform	29	Blue	0.98		6	23		
		Violet	0.97		20	9		
Chromocult® Coliform ES	21	Blue	0.96	1	6	14		
		Violet	0.95	1	10	10		
HiChrome™ Coliform	24	Blue	0.99			24		
		Yellow	0.95	1	9	14		
		Red/Violet	0.97		1	23		
HiChrome™ ECC	22	Blue	0.94	4	16	2		
		Violet	0.96		2	20		
HiChrome™ Ecoli	21	Blue	0.98	1	1	19		
		Yellow	1.00			21		
HiChrome™ Salmonella	20	Red	0.93		2	18		
		Blue	1.00			20		
HiChrome™ UTI	25	Blue	0.99			25		
		Violet	0.96		1	23	1	1
		Yellow	0.95		3	22		
HiChrome™ Listeria	27	Green	1.00			27		
RF® Listeria	21	Blue	1.00			21		
RF® Sakazakii	20	Yellow	0.97		1	19		
		Blue	0.99			20		
RF® Salmonella	20	Red	0.98			20		
		Blue	0.98			20		
BBL™ CHROMagar™ Staph aureus	26	Red	1.00			26		
		Blue	0.99			26		
BBL™ CHROMagar™ Salmonella	20	Red	0.99		9	11		
		Blue	0.78	2	4	12	2	1
BBL™ CHROMagar™ Listeria	20	Green	1.00			20		
BBL™ CHROMagar™ Orientation	21	Blue	0.93		1	20		
		Violet	0.97		8	13		
		Yellow	0.97		12	9		
		Green	0.76	2	6	12	1	
Oxoid Chromogenic Salmonella	22	Red	0.99		2	19		
		Blue	0.99			22		
Oxoid Chromogenic Listeria	22	Green	1.00			22		
Oxoid Chromogenic Sakazakii	19	Yellow	0.97	1	2	15	2	
		Blue	1.00			19		
TOTALS	**581**			**19**	**159**	**979**	**9**	**2**
				1.6%	**13.6%**	**83.8%**	**0.8%**	**0.2%**

Table 2: Summary of correlation coefficients and log differences of individual agar types

CONCLUSION

The Color QCOUNT discriminated colors and enumerated plates very well. A correlation coefficient of 0.987, a slope of 1.0067 and intercept of 0.031 all indicate a strong, linear relationship between the automated and manual methods. Log differences were within 0.2 log of the manual count for 98.2% of all plates analyzed, which is well within the standard of 0.5 log. These results demonstrate that the Color QCOUNT automated colony counter is a functional and comparable alternative to the standard method of manually counting colonies on chromogenic agar.

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Notes

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