Supporting Information

Rapid and Colorimetric Assay for Detection of *S. pneumoniae* Based on Hydrogen Peroxide Release and Analysis Using Color Image Processing

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Results and Discussion

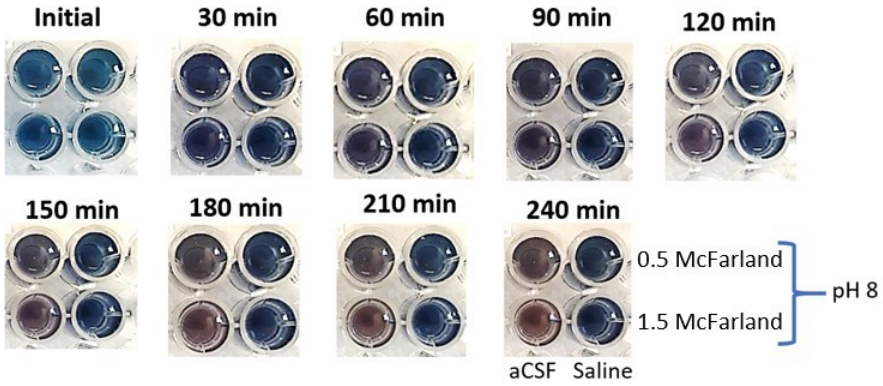

Fig. S1. Functional stability test of the anthocyanin molecule stored for 30 days. The blue color colorimetric assay was performed with *S. pneumoniae* spiked aCSF, demonstrating the successful colorimetric detection after storage. (Note: 0.5 and 1.5 McFarland correspond to $1.35x10^8$ and $4.5x10^8$ CFU/mL, respectively.)

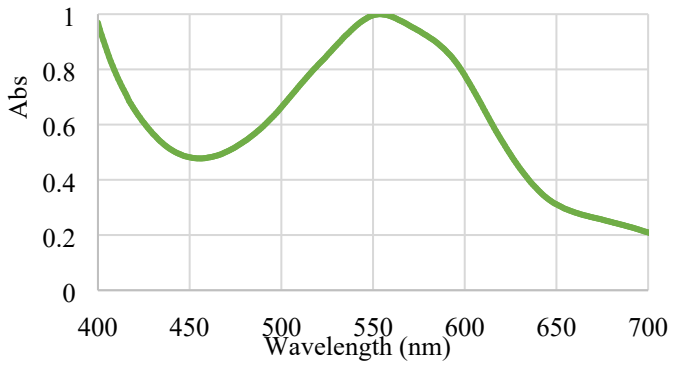

Fig. S2. UV-Vis absorption spectrum of the red cabbage extract used in the assay preparation. The spectrum exhibits a characteristic absorption maximum λmax at 550 nm, confirming the presence of anthocyanins as the primary chromophores. To ensure experimental reproducibility, the extract concentration was standardized to an absorbance of 1.0 (a.u.) at this wavelength prior to use.

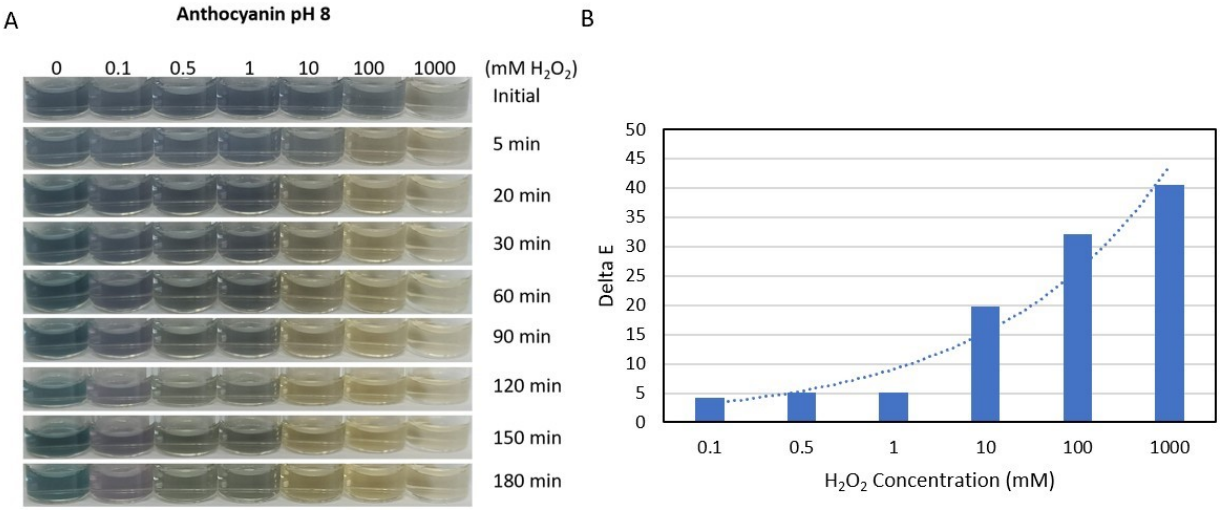

Fig. S3. Experimental validation of the sensing mechanism showing the effect of H_2O_2 concentration on anthocyanin degradation. A) Visual observation of being colorless form of anthocyanins over time (0-180 min) with increasing H_2O_2 levels. B) Quantitative dose-response analysis at 30 min. The bar graph displays the Delta E relative to the control, exhibiting an exponential increase with rising H_2O_2 concentrations (0.1 to 1000 mM). This confirms the concentration-dependent oxidative degradation of the anthocyanins.

H_2O_2

Conc. (mM)	pH
0.1	8.17
0.5	7.72
1	7.80
10	7.79
100	7.46
1000	6.40

H_2O_2 + pH 8 anthocyanin

Conc. (mM)	pH
0	7.10
0.1	7.84
0.5	5.64
1	5.59
10	6.95
100	6.59
1000	6.13

H_2O_2 + pH 11 anthocyanin

Conc. (mM)	pH
0	7.65
0.1	7.65
0.5	7.66
1	7.57
10	7.49
100	7.20
1000	6.62

Fig. S4. pH levels of H_2O_2, H_2O_2 +anthocyanin solutions at pH 8 and pH 11.

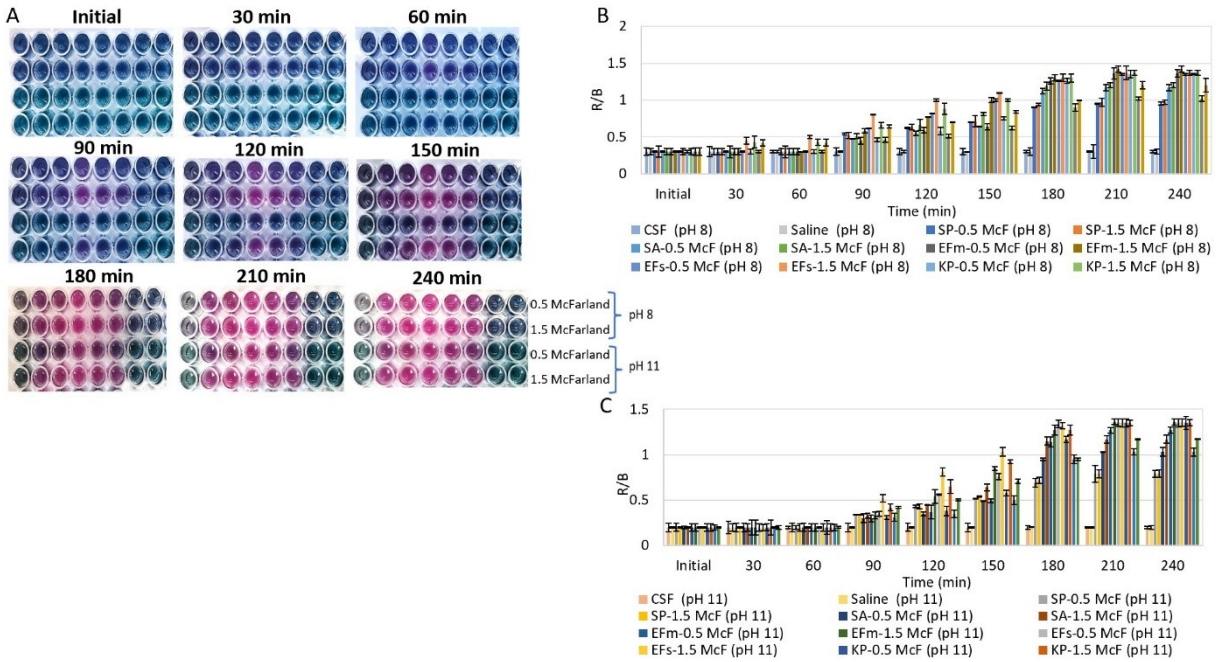

Fig. S5. A) Blue color and green colorimetric array were prepared at pH 8 and pH 11 incubated for a various period of time with different bacterial pathogens in aCSF, B) R/B analysis of blue color (pH 8) colorimetric array and, C) green color (pH 11) colorimetric array. The error bars represent standard deviation (SD) generated from three measurements (n = 3). (Note: 0.5 and 1.5 McFarland correspond to 1.35×10^8 and 4.5×10^8 CFU/mL, respectively.)

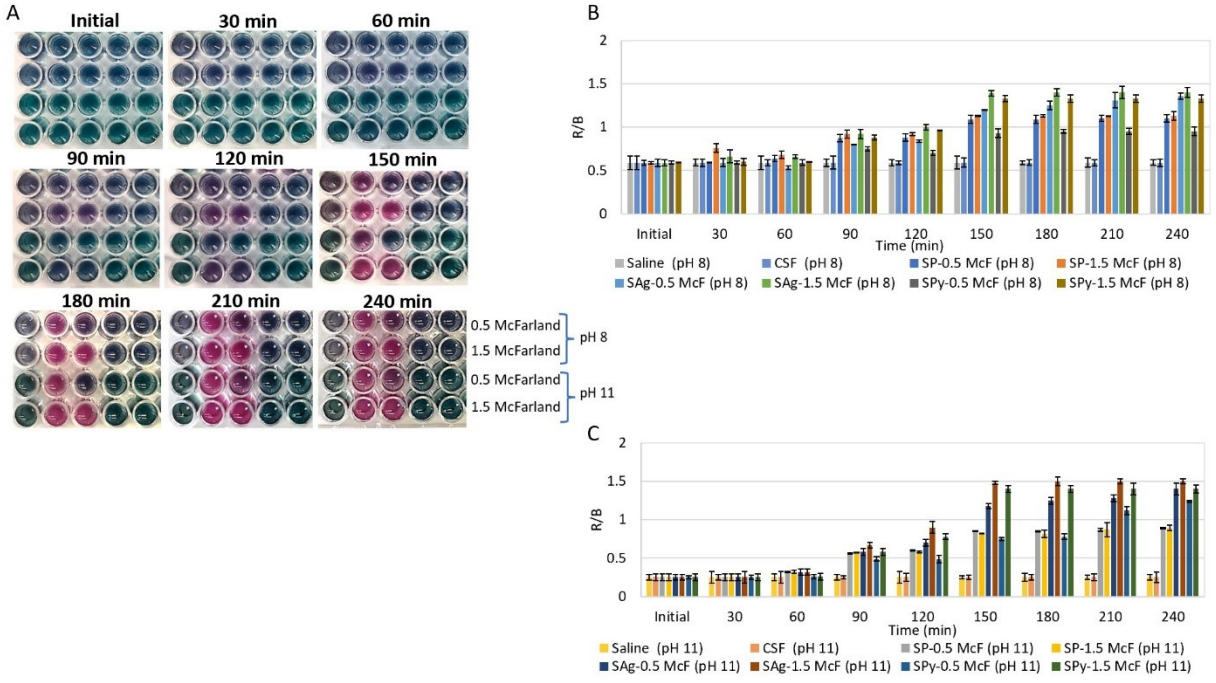

Fig. S6. A) Blue color and green colorimetric array were prepared at pH 8 and pH 11 incubated

for a various period of time with different *Streptococcus* species in aCSF, B) R/B analysis of blue color (pH 8) colorimetric array and, C) green color (pH 11) colorimetric array. The error bars represent standard deviation (SD) generated from three measurements (n = 3). (Note: 0.5 and 1.5 McFarland correspond to $1.35x10^8$ and $4.5x10^8$ CFU/mL, respectively.)

Data Analysis and Calculation of Color Difference

RGB Analysis: The Red (R), Green (G), and Blue (B) mean intensity values were extracted from the specific regions of interest (ROI) of the sensor images using ImageJ software. The primary analytical signal was defined as the normalized R/B ratio, which was subjected to rigorous statistical evaluation (Mean ± SD, %RSD) to ensure method reproducibility and minimize error propagation.

Calculation of Delta E (ΔE): To quantify the total color change, the raw RGB triplets extracted from the images were converted to the CIE Lab color space coordinates. Consistent with the methodology described in the main manuscript (Equation 1), the Delta E was calculated using the Euclidean distance metric according to the CIE 1976 standard:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½} \qquad (1)$$

- $\Delta L = L_{sample} - L_{control}$ (Difference in lightness)
- $\Delta a = a_{sample} - a_{control}$ (Difference in red-green component)
- $\Delta b = b_{sample} - b_{control}$ (Difference in yellow-blue component)

The resulting Delta E values (presented in Table S1 and S2) serve as a numerical indicator of visual perceptibility, where the variance analysis was primarily focused on the R/B ratio to ensure statistical reliability.

Table S1. Detailed raw data representing the time-dependent colorimetric array response (R/B ratio and Delta E) for *S. pneumoniae* at different concentrations (0.2-1.5 McFarland) shown in Figure 3. Data are presented as Mean ± Standard Deviation (SD) of three independent replicates (n=3), with Relative Standard Deviation (%RSD) calculated to demonstrate method reproducibility.

Time (min)	0.2 McF (Mean ±SD)	RSD %	0.5 McF (Mean± SD)	RSD %	1.0 McF (Mean± SD)	RSD%	1.5 McF (Mean± SD)	RSD %	GM (Mean± SD)	RSD%	Saline (Mean± SD)	RSD%
Initial	0.56 ± 0.05	8.9	0.58 ± 0.05	8.1	0.58 ± 0.04	6.7	0.58 ± 0.04	7.2	0.57 ± 0.02	3.7	0.57 ± 0.004	0.7
30	0.60 ± 0.027	4.5	0.61 ± 0.04	6.7	0.62 ± 0.05	8.1	0.62 ± 0.02	3.2	0.57 ± 0.05	8.1	0.57 ± 0.006	1.1
60	0.63 ± 0.01	2.1	0.65 ± 0.05	7.1	0.74 ± 0.04	5.3	0.74 ± 0.05	6.8	0.57 ± 0.01	1.6	0.57 ± 0.020	3.5
90	0.67 ± 0.05	7.2	0.68 ± 0.01	1.3	0.83 ± 0.04	5.1	0.83 ± 0.04	4.8	0.58 ± 0.05	8.4	0.58 ± 0.010	1.7
120	0.72 ± 0.04	6.1	0.74 ± 0.05	6.6	0.89 ± 0.03	3.2	0.89 ± 0.05	5.6	0.58 ± 0.01	1.0	0.58 ± 0.010	1.7
150	0.73 ± 0.05	6.3	0.74 ± 0.01	0.8	0.94 ± 0.05	5.1	0.98 ± 0.05	5.0	0.59 ± 0.004	0.7	0.59 ± 0.010	1.7
180	0.79 ± 0.02	2.5	0.82 ± 0.004	0.5	1.00 ± 0.04	3.5	1.00 ± 0.03	2.9	0.59 ± 0.06	10.2	0.59 ± 0.025	4.2
210	0.80 ± 0.05	6.3	0.84 ± 0.06	7.1	1.07 ± 0.04	4.0	1.07 ± 0.04	3.4	0.60 ± 0.02	3.3	0.60 ± 0.050	8.2
240	0.86 ± 0.04	4.7	0.94 ± 0.02	2.1	1.13 ± 0.04	3.6	1.13 ± 0.04	3.5	0.62 ± 0.01	1.6	0.62 ± 0.060	9.7

Delta E (mean)	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
0.2 McFarland-SP	8.4	9.1	9.6	10.7	12.8	15.06	22.6	23.6
0.5 McFarland-SP	8.5	9.1	9.4	10.7	12.8	15.06	22.8	24.6
1.0 McFarland-SP	8.5	9.1	13.8	13.4	19.6	20.2	24.8	25.3
1.5 McFarland-SP	8.53	9.3	13.9	13.62	19.83	20.4	24.9	25.3
GM	5.6	5.64	5.68	5.7	5.74	5.76	5.8	5.82
Saline	5.6	5.63	5.69	5.72	5.74	5.77	5.8	5.82

Table S2. Detailed raw data representing the time-dependent colorimetric array response (R/B ratio and Delta E) for *S. pneumoniae* at different concentrations (0.2-1.5 McFarland) shown in Figure 4. Data are presented as Mean ± Standard Deviation (SD) of three independent replicates (n=3), with Relative Standard Deviation (%RSD) calculated to demonstrate method reproducibility.

Time (min)	0.2 McF (Mean ±SD)	RSD %	0.5 McF (Mean± SD)	RSD%	1.0 McF (Mean± SD)	RSD %	1.5 McF (Mean± SD)	RSD %	GM (Mean± SD)	RSD%	Saline (Mean± SD)	RSD%
Initial	0.46 ± 0.025	5.43	0.46 ± 0.004	0.9	0.47 ± 0.042	8.9	0.48 ± 0.020	4.2	0.44 ± 0.041	9.3	0.44 ± 0.006	1.4
30	0.46 ± 0.007	1.5	0.46 ± 0.040	8.7	0.47 ± 0.041	8.7	0.48 ± 0.040	8.3	0.44 ± 0.046	10.5	0.44 ± 0.004	0.9
60	0.48 ± 0.013	2.7	0.49 ± 0.040	8.2	0.49 ± 0.018	3.7	0.50 ± 0.040	8.0	0.45 ± 0.009	2.0	0.45 ± 0.010	2.2
90	0.49 ± 0.048	9.8	0.50 ± 0.005	1.0	0.52 ± 0.035	6.7	0.54 ± 0.019	3.5	0.46 ± 0.005	1.1	0.46 ± 0.020	4.3
120	0.50 ± 0.044	8.8	0.50 ± 0.005	1.0	0.53 ± 0.011	2.1	0.56 ± 0.010	1.8	0.46 ± 0.008	1.7	0.46 ± 0.010	2.2
150	0.51 ± 0.046	9.0	0.51 ± 0.003	0.6	0.53 ± 0.015	2.8	0.57 ± 0.021	3.7	0.47 ± 0.005	1.1	0.47 ± 0.010	2.1
180	0.56 ± 0.020	3.6	0.57 ± 0.006	1.1	0.58 ± 0.049	8.4	0.62 ± 0.060	9.7	0.49 ± 0.004	0.8	0.49 ± 0.025	5.1
210	0.68 ± 0.050	7.4	0.68 ± 0.004	0.6	0.71 ± 0.029	4.1	0.76 ± 0.050	6.6	0.51 ± 0.010	2.0	0.51 ± 0.015	2.9
240	0.71 ± 0.014	2.0	0.75 ± 0.017	2.3	0.78 ± 0.036	4.6	0.82 ± 0.050	6.1	0.51 ± 0.012	2.4	0.51 ± 0.016	3.13

Delta E (mean)	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
0.2 McFarland-SP	2.8	2.8	2.8	2.81	2.83	2.84	5.01	5.11
0.5 McFarland-SP	2.8	282	2.82	2.85	2.88	2.89	6.5	6.54
1.0 McFarland-SP	2.8	2.81	2.83	2.85	2.86	2.89	10.7	10.75
1.5 McFarland-SP	2.8	2.8	2.8	2.8	2.8	2.8	10.7	10.77
GM	2.9	2.9	2.94	2.96	2.97	2.97	2.98	2.98
Saline	2.7	2.74	2.77	2.79	2.8	2.82	2.83	2.83

Table S3. Detailed numerical data for bacterial selectivity experiments at pH 8 shown in Figure 5. The table lists the colorimetric array response (Mean R/B ratio) for various bacterial strains (*S. pneumoniae, S. aureus, E. faecium, E. faecalis, K. pneumoniae, E. coli*) at 0.5 and 1.5

McFarland concentrations. Values represent Mean ± SD (n=3), and %RSD is provided to verify the precision of the measurements.

Time (min)	Saline (Control)	SP 0.5 McF	SP 1.5 McF	SA 0.5 McF	SA 1.5 McF	EFm 0.5 McF
Initial	0.75 ± 0.009 (1.2%)	0.75 ± 0.050 (6.7%)	0.75 ± 0.004 (0.5%)	0.75 ± 0.048 (6.4%)	0.75 ± 0.020 (2.7%)	0.75 ± 0.028 (3.7%)
30	0.75 ± 0.005 (0.7%)	0.75 ± 0.070 (9.3%)	0.82 ± 0.006 (0.7%)	0.75 ± 0.050 (6.7%)	0.75 ± 0.010 (1.3%)	0.75 ± 0.024 (3.2%)
60	0.75 ± 0.006 (0.8%)	0.75 ± 0.013 (1.7%)	0.85 ± 0.020 (2.4%)	0.75 ± 0.019 (2.5%)	0.75 ± 0.013 (1.7%)	0.78 ± 0.028 (3.6%)
90	0.75 ± 0.004 (0.5%)	0.78 ± 0.048 (6.2%)	0.91 ± 0.010 (1.1%)	0.75 ± 0.017 (2.3%)	0.75 ± 0.015 (2.0%)	0.88 ± 0.039 (4.4%)
120	0.75 ± 0.006 (0.8%)	0.78 ± 0.044 (5.6%)	0.93 ± 0.010 (1.1%)	0.75 ± 0.032 (4.3%)	0.77 ± 0.018 (2.3%)	1.14 ± 0.021 (1.8%)
150	0.75 ± 0.020 (2.7%)	0.90 ± 0.046 (5.1%)	0.98 ± 0.010 (1.0%)	0.75 ± 0.021 (2.8%)	0.78 ± 0.019 (2.4%)	1.14 ± 0.031 (2.7%)
180	0.75 ± 0.010 (1.3%)	0.90 ± 0.020 (2.2%)	0.98 ± 0.027 (2.8%)	0.75 ± 0.028 (3.7%)	0.78 ± 0.011 (1.4%)	1.14 ± 0.050 (4.4%)
210	0.75 ± 0.010 (1.3%)	0.97 ± 0.005 (0.5%)	1.03 ± 0.012 (1.2%)	0.81 ± 0.049 (6.0%)	0.94 ± 0.004 (0.4%)	1.14 ± 0.012 (1.1%)
240	0.75 ± 0.010 (1.3%)	0.97 ± 0.014 (1.4%)	1.03 ± 0.006 (0.6%)	0.81 ± 0.021 (2.6%)	0.94 ± 0.006 (0.6%)	1.14 ± 0.017 (1.5%)

Table S3 (continued). Detailed numerical data for bacterial selectivity experiments at pH 8 shown in Figure 5. The table lists the colorimetric array response (Mean R/B ratio) for various bacterial strains (*S. pneumoniae, S. aureus, E. faecium, E. faecalis, K. pneumoniae, E. coli*) at 0.5 and 1.5 McFarland concentrations. Values represent Mean ± SD (n=3), and %RSD is provided to verify the precision of the measurements.

Time (min)	EFm 1.5 McF	EFs 0.5 McF	EFs 1.5 McF	KP 0.5 McF	KP 1.5 McF	EC 0.5 McF	EC 1.5 McF
Initial	0.75 ± 0.037 (4.9%)	0.75 ± 0.012 (1.6%)	0.75 ± 0.025 (3.3%)	0.75 ± 0.013 (1.7%)	0.75 ± 0.029 (3.9%)	0.75 ± 0.030 (4.0%)	0.75 ± 0.043 (5.7%)
30	0.77 ± 0.051 (6.6%)	0.75 ± 0.011 (1.5%)	0.75 ± 0.038 (5.1%)	0.75 ± 0.031 (4.1%)	0.75 ± 0.045 (6.0%)	0.75 ± 0.051 (6.8%)	0.75 ± 0.016 (2.1%)
60	0.83 ± 0.015 (1.8%)	0.75 ± 0.007 (0.9%)	0.75 ± 0.013 (1.7%)	0.75 ± 0.021 (2.8%)	0.78 ± 0.017 (2.2%)	0.75 ± 0.022 (2.9%)	0.78 ± 0.015 (1.9%)
90	1.00 ± 0.015 (1.5%)	0.75 ± 0.015 (2.0%)	0.75 ± 0.011 (1.5%)	0.75 ± 0.032 (4.3%)	0.89 ± 0.022 (2.5%)	0.75 ± 0.034 (4.5%)	0.82 ± 0.028 (3.4%)
120	1.16 ± 0.029 (2.5%)	0.80 ± 0.011 (1.4%)	0.88 ± 0.025 (2.8%)	0.86 ± 0.021 (2.4%)	1.02 ± 0.034 (3.3%)	0.83 ± 0.029 (3.5%)	1.00 ± 0.031 (3.1%)
150	1.16 ± 0.029 (2.5%)	0.82 ± 0.015 (1.8%)	1.11 ± 0.027 (2.4%)	0.96 ± 0.019 (2.0%)	1.09 ± 0.021 (1.9%)	0.82 ± 0.027 (3.3%)	1.07 ± 0.027 (2.5%)
180	1.16 ± 0.024	1.00 ± 0.010	1.12 ± 0.021	1.04 ± 0.023	1.10 ± 0.028	0.92 ± 0.015	1.09 ± 0.044

	(2.1%)	(1.0%)	(1.9%)	(2.2%)	(2.5%)	(1.6%)	(4.0%)
210	1.16 ± 0.025 (2.2%)	1.15 ± 0.010 (0.9%)	1.25 ± 0.043 (3.4%)	1.15 ± 0.020 (1.7%)	1.25 ± 0.031 (2.5%)	1.04 ± 0.015 (1.4%)	1.14 ± 0.017 (1.5%)
240	1.16 ± 0.024 (2.1%)	1.15 ± 0.023 (2.0%)	1.25 ± 0.028 (2.2%)	1.15 ± 0.020 (1.7%)	1.25 ± 0.023 (1.8%)	1.04 ± 0.013 (1.3%)	1.14 ± 0.015 (1.3%)

Table S4. Detailed numerical data for bacterial selectivity experiments at pH 11 shown in Figure 5. The table lists the colorimetric array response (Mean R/B ratio) for various bacterial strains (*S. pneumoniae, S. aureus, E. faecium, E. faecalis, K. pneumoniae, E. coli*) at 0.5 and 1.5 McFarland concentrations. Values represent Mean ± SD (n=3), and %RSD is provided to verify the precision of the measurements.

Time (min)	Saline (Control)	SP 0.5 McF	SP 1.5 McF	SA 0.5 McF	SA 1.5 McF	EFm 0.5 McF
Initial	0.60 ± 0.053 (8.8%)	0.60 ± 0.006 (1.0%)	0.60 ± 0.013 (2.2%)	0.60 ± 0.038 (6.33%)	0.60 ± 0.004 (0.7%)	0.60 ± 0.042 (7.0%)
30	0.60 ± 0.015 (2.5%)	0.60 ± 0.005 (0.8%)	0.60 ± 0.006 (1.0%)	0.60 ± 0.040 (6.7%)	0.60 ± 0.035 (5.8%)	0.60 ± 0.045 (7.5%)
60	0.60 ± 0.039 (6.5%)	0.60 ± 0.039 (6.5%)	0.67 ± 0.012 (1.8%)	0.60 ± 0.043 (7.16%)	0.60 ± 0.040 (6.7%)	0.60 ± 0.024 (4.0%)
90	0.60 ± 0.039 (6.5%)	0.60 ± 0.004 (0.6%)	0.67 ± 0.011 (1.6%)	0.60 ± 0.042 (7.0%)	0.60 ± 0.041 (6.8%)	0.66 ± 0.019 (2.9%)
120	0.60 ± 0.047 (7.8%)	0.63 ± 0.031 (4.9%)	0.69 ± 0.002 (0.3%)	0.60 ± 0.037 (6.2%)	0.63 ± 0.045 (7.1%)	0.91 ± 0.029 (3.2%)
150	0.60 ± 0.023 (3.8%)	0.66 ± 0.005 (0.8%)	0.77 ± 0.010 (1.3%)	0.60 ± 0.025 (4.16%)	0.63 ± 0.048 (7.6%)	1.08 ± 0.025 (2.3%)
180	0.60 ± 0.021 (3.5%)	0.66 ± 0.035 (5.3%)	0.77 ± 0.030 (3.9%)	0.61 ± 0.020 (3.3%)	0.65 ± 0.031 (4.8%)	1.11 ± 0.025 (2.3%)
210	0.60 ± 0.017 (2.8%)	0.76 ± 0.041 (5.4%)	0.82 ± 0.018 (2.2%)	0.64 ± 0.054 (8.4%)	0.71 ± 0.040 (5.6%)	1.11 ± 0.041 (3.7%)
240	0.60 ± 0.017 (2.8%)	0.76 ± 0.023 (3.0%)	0.82 ± 0.010 (1.2%)	0.64 ± 0.031 (4.8%)	0.71 ± 0.040 (5.6%)	1.11 ± 0.041 (3.7%)

Table S4 (continued). Detailed numerical data for bacterial selectivity experiments at pH 11 shown in Figure 5. The table lists the colorimetric array response (Mean R/B ratio) for various bacterial strains (*S. pneumoniae, S. aureus, E. faecium, E. faecalis, K. pneumoniae, E. coli*) at 0.5 and 1.5 McFarland concentrations. Values represent Mean ± SD (n=3), and %RSD is provided to verify the precision of the measurements.

Time	EFm 1.5 McF	EFs 0.5 McF	EFs 1.5 McF	KP 0.5 McF	KP 1.5 McF	EC 0.5 McF	EC 1.5 McF

(min)							
Initial	0.60 ± 0.045 (7.5%)	0.60 ± 0.048 (8.0%)	0.60 ± 0.050 (8.3%)	0.60 ± 0.004 (0.7%)	0.60 ± 0.040 (6.7%)	0.60 ± 0.038 (6.3%)	0.60 ± 0.017 (2.8%)
30	0.60 ± 0.044 (7.33%)	0.60 ± 0.050 (8.3%)	0.60 ± 0.051 (8.5%)	0.60 ± 0.036 (6.0%)	0.60 ± 0.030 (5%)	0.60 ± 0.042 (7.0%)	0.60 ± 0.032 (5.3%)
60	0.69 ± 0.017 (2.5%)	0.60 ± 0.019 (3.2%)	0.60 ± 0.013 (2.2%)	0.60 ± 0.005 (0.8%)	0.66 ± 0.042 (6.4%)	0.60 ± 0.037 (6.2%)	0.66 ± 0.026 (3.9%)
90	0.81 ± 0.037 (4.6%)	0.60 ± 0.018 (3.0%)	0.60 ± 0.048 (8.0%)	0.60 ± 0.035 (5.8%)	0.68 ± 0.037 (5.4%)	0.60 ± 0.035 (5.83%)	0.68 ± 0.028 (4.1%)
120	1.08 ± 0.042 (3.9%)	0.62 ± 0.032 (5.2%)	0.69 ± 0.044 (6.4%)	0.69 ± 0.039 (5.7%)	0.89 ± 0.072 (8.1%)	0.69 ± 0.020 (2.9%)	0.89 ± 0.049 (5.5%)
150	1.09 ± 0.042 (3.9%)	0.76 ± 0.019 (2.5%)	0.86 ± 0.046 (5.3%)	0.81 ± 0.041 (5.1%)	1.05 ± 0.020 (1.9%)	0.80 ± 0.054 (6.8%)	1.05 ± 0.021 (2.0%)
180	1.13 ± 0.015 (1.3%)	0.89 ± 0.028 (3.1%)	1.05 ± 0.020 (1.9%)	1.00 ± 0.045 (4.5%)	1.12 ± 0.063 (5.6%)	0.82 ± 0.031 (3.8%)	1.07 ± 0.035 (3.3%)
210	1.13 ± 0.035 (3.1%)	1.13 ± 0.049 (4.3%)	1.27 ± 0.005 (0.4%)	1.13 ± 0.043 (3.8%)	1.27 ± 0.021 (1.7%)	0.99 ± 0.035 (3.5%)	1.18 ± 0.050 (4.2%)
240	1.13 ± 0.021 (1.9%)	1.13 ± 0.021 (1.9%)	1.27 ± 0.014 (1.1%)	1.13 ± 0.025 (2.2%)	1.27 ± 0.035 (2.8%)	0.99 ± 0.042 (4.2%)	1.18 ± 0.009 (0.8%)

Table S5. Raw data comparison of streptococcal strains (*S. pneumoniae, S. agalactiae, S. pyogenes*) at pH 8 shown in Figure 6. The table details the time-dependent colorimetric response (Mean R/B) over 240 minutes. Statistical parameters (Mean ± SD, %RSD) are included to demonstrate intra-assay reproducibility (n=3).

Time (min)	Saline (Control)	SP 0.5 McF	SP 1.5 McF	Sag 0.5 McF	Sag 1.5 McF	SPy 0.5 McF	SPy 1.5 McF
Initial	0.47 ± 0.041 (8.7%)	0.47 ± 0.006 (1.3%)	0.47 ± 0.019 (4.0%)	0.47 ± 0.039 (8.3%)	0.47 ± 0.021 (4.5%)	0.47 ± 0.040 (8.5%)	0.47 ± 0.035 (7.44%)
30	0.47 ± 0.046 (9.8%)	0.47 ± 0.005 (1.1%)	0.66 ± 0.017 (2.6%)	0.47 ± 0.004 (0.9%)	0.61 ± 0.035 (5.7%)	0.47 ± 0.021 (4.5%)	0.54 ± 0.028 (5.18%)
60	0.47 ± 0.009 (1.9%)	0.57 ± 0.039 (6.8%)	0.67 ± 0.032 (4.8%)	0.55 ± 0.029 (5.3%)	0.66 ± 0.050 (7.6%)	0.55 ± 0.021 (3.8%)	0.57 ± 0.013 (2.3%)
90	0.47 ± 0.005 (1.0%)	0.57 ± 0.004 (0.7%)	0.70 ± 0.021 (3.0%)	0.65 ± 0.004 (0.6%)	0.89 ± 0.009 (1.0%)	0.54 ± 0.029 (5.4%)	0.69 ± 0.048 (7.0%)
120	0.47 ± 0.006 (1.3%)	0.60 ± 0.026 (4.3%)	0.73 ± 0.028 (3.8%)	0.81 ± 0.035 (4.3%)	1.05 ± 0.070 (6.7%)	0.57 ± 0.025 (4.4%)	0.75 ± 0.044 (5.9%)
150	0.47 ± 0.004 (0.9%)	0.67 ± 0.004 (0.6%)	0.79 ± 0.049 (6.2%)	1.00 ± 0.043 (4.3%)	1.12 ± 0.032 (2.9%)	0.84 ± 0.034 (4.0%)	1.00 ± 0.046 (4.6%)
180	0.47 ± 0.006 (1.3%)	0.69 ± 0.035 (5.1%)	0.82 ± 0.021 (2.6%)	1.12 ± 0.041 (3.7%)	1.14 ± 0.068 (6.0%)	0.95 ± 0.042 (4.4%)	1.00 ± 0.020 (2.0%)
210	0.47 ± 0.020 (4.3%)	0.69 ± 0.043 (6.2%)	0.82 ± 0.035 (4.3%)	1.12 ± 0.045 (4.0%)	1.14 ± 0.080 (7.0%)	1.02 ± 0.033 (3.2%)	1.02 ± 0.005 (0.5%)
240	0.47 ± 0.010 (2.1%)	0.69 ± 0.041 (5.9%)	0.82 ± 0.050 (6.1%)	1.12 ± 0.045 (4.0%)	1.14 ± 0.006 (0.5%)	1.02 ± 0.024 (2.4%)	1.02 ± 0.014 (1.4%)

Table S6. Raw data comparison of streptococcal strains at pH 1 shown in Figure 6. This dataset

highlights the differential colorimetric array response to specific streptococcal species under alkaline conditions. All data points represent the average of independent triplicates (Mean ± SD) with calculated precision (%RSD).

Time (min)	Saline (Control)	SP 0.5 McF	SP 1.5 McF	Sag 0.5 McF	Sag 1.5 McF	SPy 0.5 McF	SPy 1.5 McF
Initial	0.30 ± 0.017 (5.7%)	0.30 ± 0.027 (9.0%)	0.30 ± 0.018 (6.0%)	0.30 ± 0.004 (1.3%)	0.30 ± 0.005 (1.6%)	0.30 ± 0.018 (6.0%)	0.30 ± 0.010 (3.3%)
30	0.30 ± 0.025 (8.3%)	0.30 ± 0.025 (8.3%)	0.30 ± 0.027 (9.0%)	0.30 ± 0.026 (8.6%)	0.30 ± 0.017 (5.7%)	0.30 ± 0.021 (7.0%)	0.30 ± 0.028 (9.3%)
60	0.30 ± 0.028 (9.3%)	0.42 ± 0.020 (4.8%)	0.46 ± 0.019 (4.1%)	0.45 ± 0.005 (1.1%)	0.49 ± 0.004 (0.8%)	0.44 ± 0.038 (8.6%)	0.47 ± 0.041 (8.7%)
90	0.30 ± 0.023 (7.6%)	0.42 ± 0.022 (5.2%)	0.46 ± 0.023 (5.0%)	0.53 ± 0.031 (5.8%)	0.66 ± 0.005 (0.8%)	0.44 ± 0.042 (9.5%)	0.48 ± 0.027 (5.6%)
120	0.30 ± 0.029 (9.6%)	0.42 ± 0.031 (7.4%)	0.46 ± 0.031 (6.7%)	0.64 ± 0.041 (6.4%)	1.00 ± 0.020 (2.0%)	0.44 ± 0.038 (8.6%)	0.55 ± 0.016 (2.9%)
150	0.30 ± 0.021 (7.0%)	0.42 ± 0.035 (8.3%)	0.46 ± 0.027 (5.9%)	0.84 ± 0.045 (5.4%)	1.12 ± 0.026 (2.3%)	0.44 ± 0.035 (7.9%)	0.84 ± 0.006 (0.7%)
180	0.30 ± 0.017 (5.6%)	0.43 ± 0.042 (9.8%)	0.48 ± 0.028 (5.8%)	1.02 ± 0.039 (3.8%)	1.15 ± 0.033 (2.9%)	0.47 ± 0.020 (4.3%)	0.93 ± 0.004 (0.4%)
210	0.30 ± 0.021 (7.0%)	0.43 ± 0.031 (7.2%)	0.48 ± 0.045 (9.4%)	1.02 ± 0.045 (4.4%)	1.15 ± 0.026 (2.3%)	0.95 ± 0.054 (5.7%)	1.01 ± 0.006 (0.6%)
240	0.30 ± 0.009 (3.0%)	0.43 ± 0.027 (6.3%)	0.48 ± 0.031 (6.5%)	1.02 ± 0.023 (2.3%)	1.15 ± 0.021 (1.8%)	0.95 ± 0.041 (4.3%)	1.01 ± 0.040 (4.0%)

All data represent Mean ± Standard Deviation (RSD%) of independent triplicates (n=3).

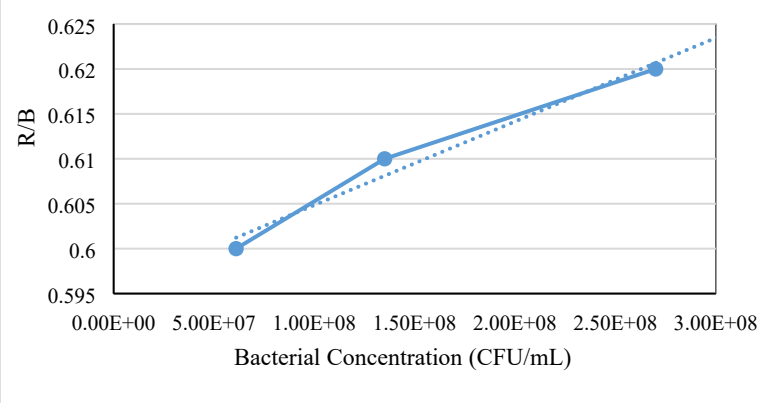

Fig. S7. Linear calibration graph of the colorimetric array response (R/B ratio) versus bacterial concentration at pH 8 after 30 min incubation. The regression equation (y = 9E-11x + 0.5955) and coefficient of determination (R^2 = 0.9724) were used for the calculation of LOD/LOQ.

Table S7. Calibration parameters and analytical limits (LOD/LOQ) calculated for the pH 8 anthocyanin formulation.

Incubation Time (min)	σ_{blank}	Slope (S)	R^2	LOD ($3\sigma/S$)	LOQ ($10\sigma/S$)
30	0.006	9.00×10^{-11}	0.97	2.00×10^{8}	6.67×10^{8}
90	0.010	8.00×10^{-10}	0.91*	3.75×10^{7}	1.25×10^{8}

LOD and LOQ were calculated based on the standard deviation of the blank (σ) and the slope (S) of the linear regression curve. Note: While the 30 min assay provides optimal linearity (R^2=0.97) for quantitative analysis, extending the time to 90 min enables higher sensitivity (lower LOD), albeit with a reduction in linearity due to signal saturation effects.

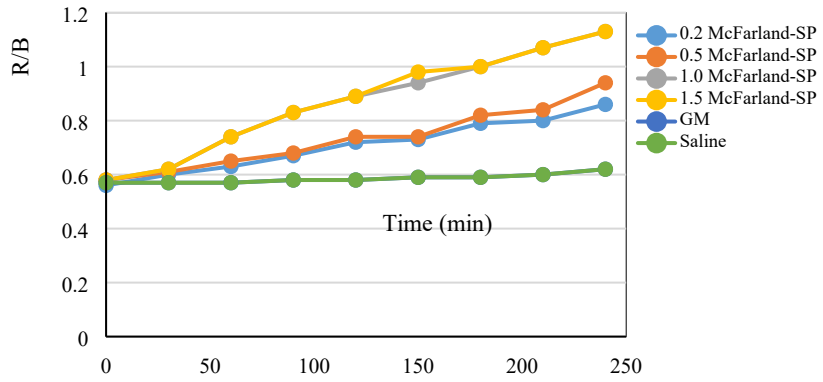

Fig. S8. Kinetic analysis of the colorimetric array response over time for different bacterial concentrations (0.2-1.5 McFarland). The curves demonstrate a time-dependent signal amplification, where higher bacterial loads induce rapid color changes within 30 min, whereas lower concentrations require extended incubation to generate distinct signals above the baseline.

Table S8. Comparison of the proposed colorimetric assay with traditional diagnostic methods for *S. pneumoniae*.

Diagnostic Method	Time to Result	Estimated Cost per Test	Readout Strategy	Expertise Required	Ref.
Anthocyanin based Colorimetric Assay	30 - 90 min	Very Low	Digital Image Processing (Objective)	Minimal (Automated analysis)	This work
Microbial Culture (Gold Standard)	2 - 5 days	Low-Medium (High labor cost)	Visual Inspection (Subjective)	High (Microbiologist)	39
Molecular Methods (e.g., PCR)	2 - 4 hours	High	Ct Values / Electrophoresis	High (Molecular Biologist)	40
Immunological Tests (e.g., ELISA)	3 - 5 hours	High	Optical Density / Fluorescence	Medium (Lab Technician)	41

Table S8 summarises the typical time-to-result, readout strategy and expertise requirements of common diagnostic modalities, in order to inform the evaluation of the proposed assay. The values represent commonly reported ranges in the literature and are intended for qualitative comparison rather than exhaustive cost analysis.

Table S9. Performance comparison of the developed anthocyanin-based colorimetric assay with recently published optical biosensors for *S. pneumoniae* detection.

Sensing Probe / Material	Detection Principle	Sample Matrix	LOD	Response Time	Cost & Complexity	Ref.
Anthocyanin (Red Cabbage)	Colorimetric (Oxidative Degradation)	aCSF, Saline	~ 3.75×10^7 CFU/mL	30 - 90 min	Low (natural pigment; no proprietary substrates; optional image analysis)	This Work
GO–AuNP/QD "dual-signal" ICA tag	Colorimetric + fluorescence immunochromatography (ICA)	Real biological + environmental samples	17 cells/mL (for *S. pneumoniae*)	≤ 20 min	Medium (nanomaterial tag + strip)	42
SiO_2@Au "core–satellite" SERS tag (ICA)	SERS-ICA (quantitative optical readout)	Respiratory tract samples / complex samples	46 cells/mL (IUPAC LOD)	~20 min (chromatography optimized 15 min)	Medium–High (SERS + strip)	43
WGA-modified magnetic SERS nanotags (LFA)	SERS–LFA (optical Raman readout; dual-channel)	Throat swab / sputum (clinical)	10 cells/mL	40 min	High (magnetic SERS tags + Raman)	44
Oligonucleotide probe (nuclease biomarker)	Fluorescence biosensing (optical)	Cultured isolates / clinical isolates	10^6 CFU/mL (fluorescence modu)	15–60 min	Medium (fluor + probe)	45
Microfluidic Au@Pt nanozyme immunosensor	Nanozyme-catalyzed colorimetry (TMB oxidation) + smartphone	Saliva	21 CFU/mL	~20 min	Medium–High (chip + nanozyme + other steps)	46

The comparison highlights the trade-off between analytical sensitivity and practical applicability. Recent methods utilizing SERS tags or dual-signal probes offer high sensitivity (low LOD) suitable for trace analysis but involve high costs and complex synthesis steps.[42-44] The proposed assay, utilizing natural anthocyanins, provides a "low-cost, synthesis-free" alternative. Although the LOD is higher, it is sufficient for the rapid confirmation of high bacterial loads found in cultured isolates or clinical specimens from acute infections, minimizing the need for advanced infrastructure.