SUPPPORTING INFORMATION

Rapid detection of major Gram-positive pathogens in ocular specimens using a novel fluorescent vancomycin-based probe

Richa Sharma[a], Harinee Rajagopalan[b], Maxime Klausen[a], Mani Vimalin Jeyalatha[b], Muhammed Üçüncü[a,c] , Seshasailam Venkateswaran[a], Appakkudal R Anand[b*] and Mark Bradley[a] *

[a]EaStCHEM, School of Chemistry, King's Buildings, University of Edinburgh, Edinburgh, EH9 3FJ, UK
[b]L & T Microbiology Research Centre, Medical Research Foundation, Sankara Nethralaya, Chennai, India
[c]Department of Analytical Chemistry, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey

1. Chemistry

Reagents and experimental methods

Commercially available reagents were used without further purification. NMR spectra were recorded using Bruker AC spectrometers operating at 500 MHz for ^{1}H and ^{13}C NMR. Chemical shifts are reported on the δ scale in ppm and are referenced to residual non-deuterated solvent resonances. Normal phase purifications by column chromatography were carried out on silica gel 60 (230-400 mesh). Analytical reverse-phase high-performance liquid chromatography (RP–HPLC) was performed on an Agilent 1100 system equipped with a Phenomenex Kinetex® 5 µm XB-C18 100 Å LC Column (50 × 4.6 mm) with a flow rate of 1 mL/min. Method 1: A gradient of H_2O/CH_3CN (95/5) to H_2O/CH_3CN (5/95) with 0.1% HCOOH, over 6 min, holding at 95% CH_3CN for 3 min, followed by 1 min isocratic elution. Method 2: A gradient of H_2O/CH_3CN (95/5) to H_2O/CH_3CN (20/80) with 0.1% CF_3COOH, over 10 min, then to H_2O/CH_3CN (5/95), over 4 min, followed by 1 min isocratic elution with detection at 495 nm and by evaporative light scattering. Preparative RP–HPLC was performed on an Agilent 1100 system equipped with a Phenomenex Kinetex® 5µm XB-C18 reverse-phase column (150 × 21.2 mm, 5 µm) with a flow rate of 10 mL/min. A gradient of H_2O/CH_3CN (80/20) to H_2O/CH_3CN (50/50) with 0.1% CF_3COOH, over 15 min then from H_2O/CH_3CN (50/50) to H_2O/CH_3CN (5/95) over 4 min, followed by 1 min isocratic elution with detection at 495 nm. Electrospray ionization mass spectrometry (ESI–MS) analyses were carried out on an Agilent Technologies LC/MSD Series 1100 quadrupole mass spectrometer (QMS) in an ESI mode. High Resolution MS were performed on a Bruker microTOF focus II mass spectrometer. Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer.

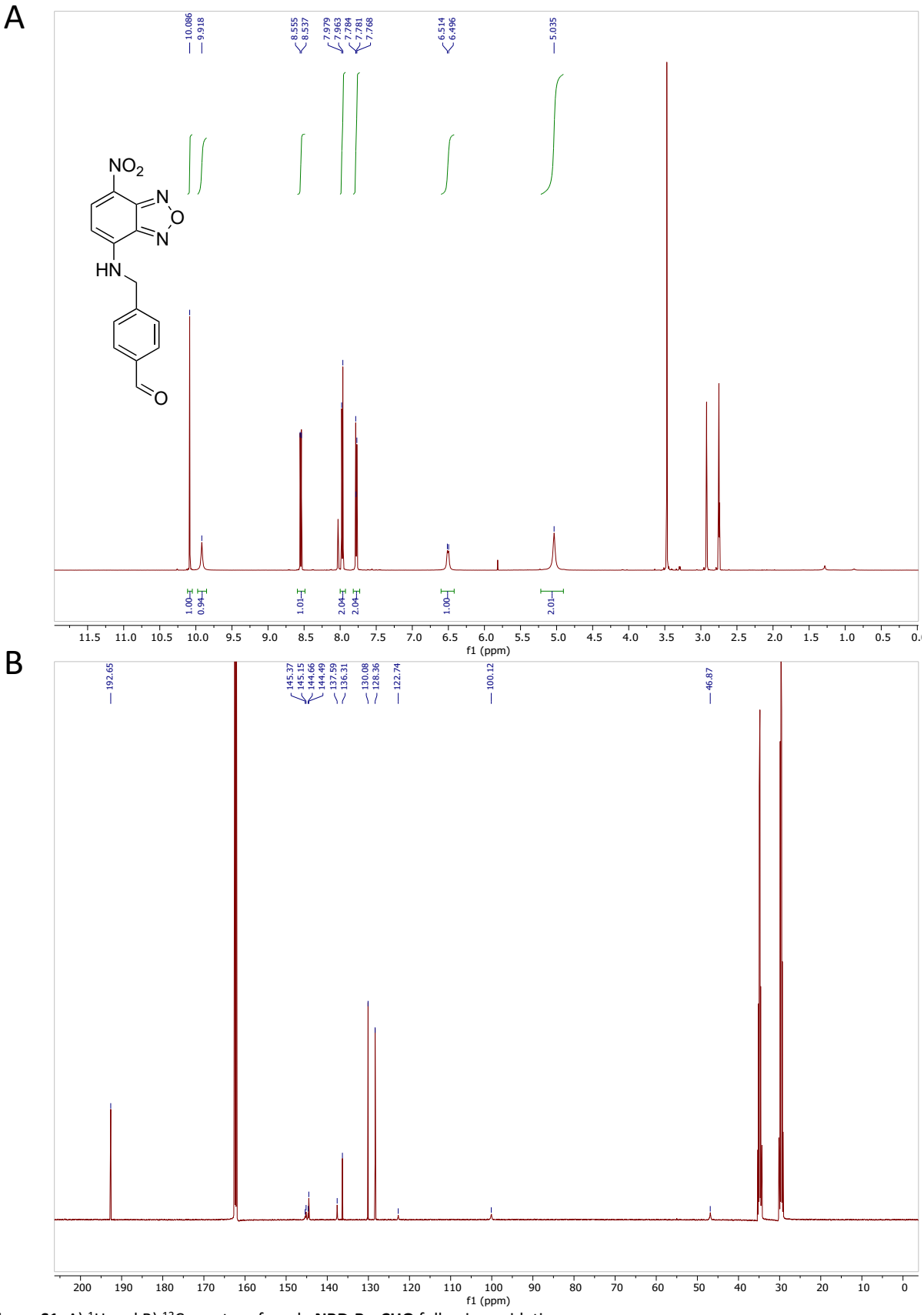


Figure S1. A) ^{1}H and B) ^{13}C spectra of crude **NBD-Bn-CHO** following oxidation.

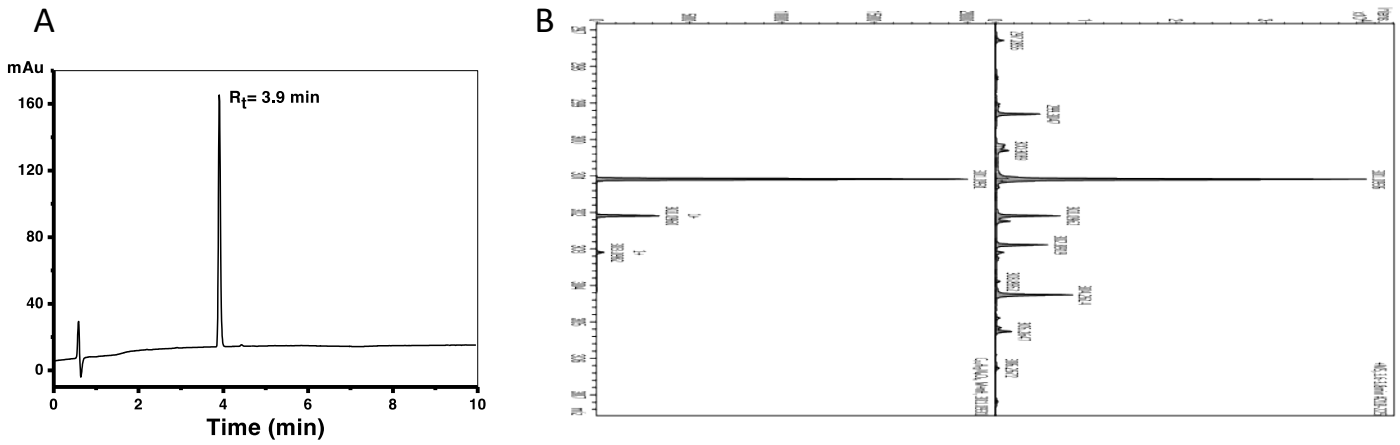


Figure S2. A) RP-HPLC Analysis of compound **1** (NBD-Bn-OH) (detection at 495 nm) and **B)** HRMS traces of compound **NBD-Bn-OH (**left panel: theoretical $C_{14}H_{12}N_4O_4$, right panel: experimental)

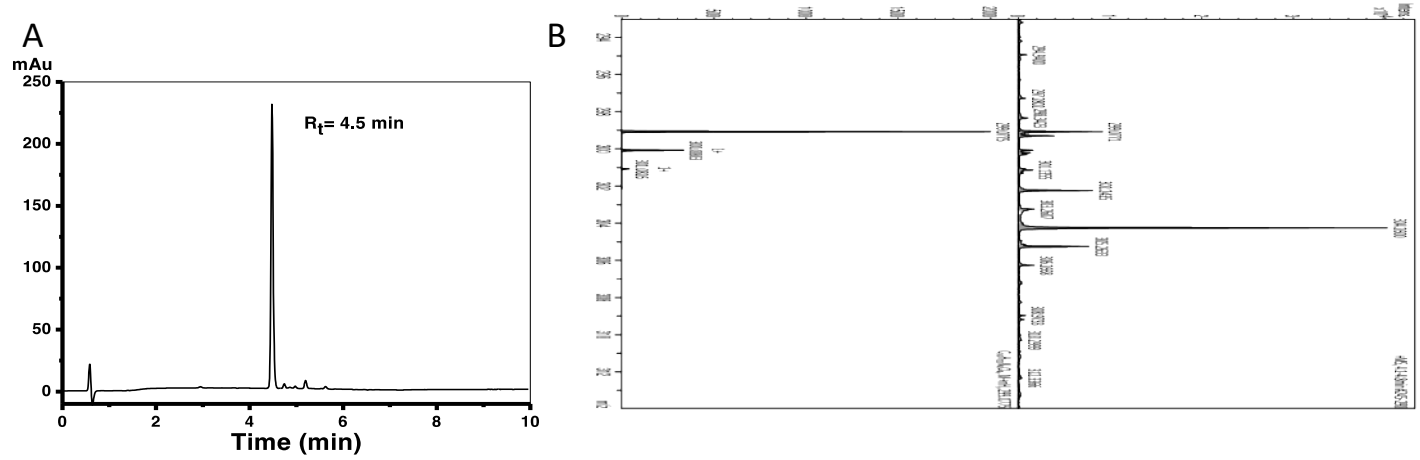


Figure S3. A) RP-HPLC Analysis of compound **2** (detection at 495 nm) and **B)** HRMS traces of compound **NBD-Bn-CHO (**left panel: theoretical $C_{14}H_{10}N_4O_4$, right panel: experimental)

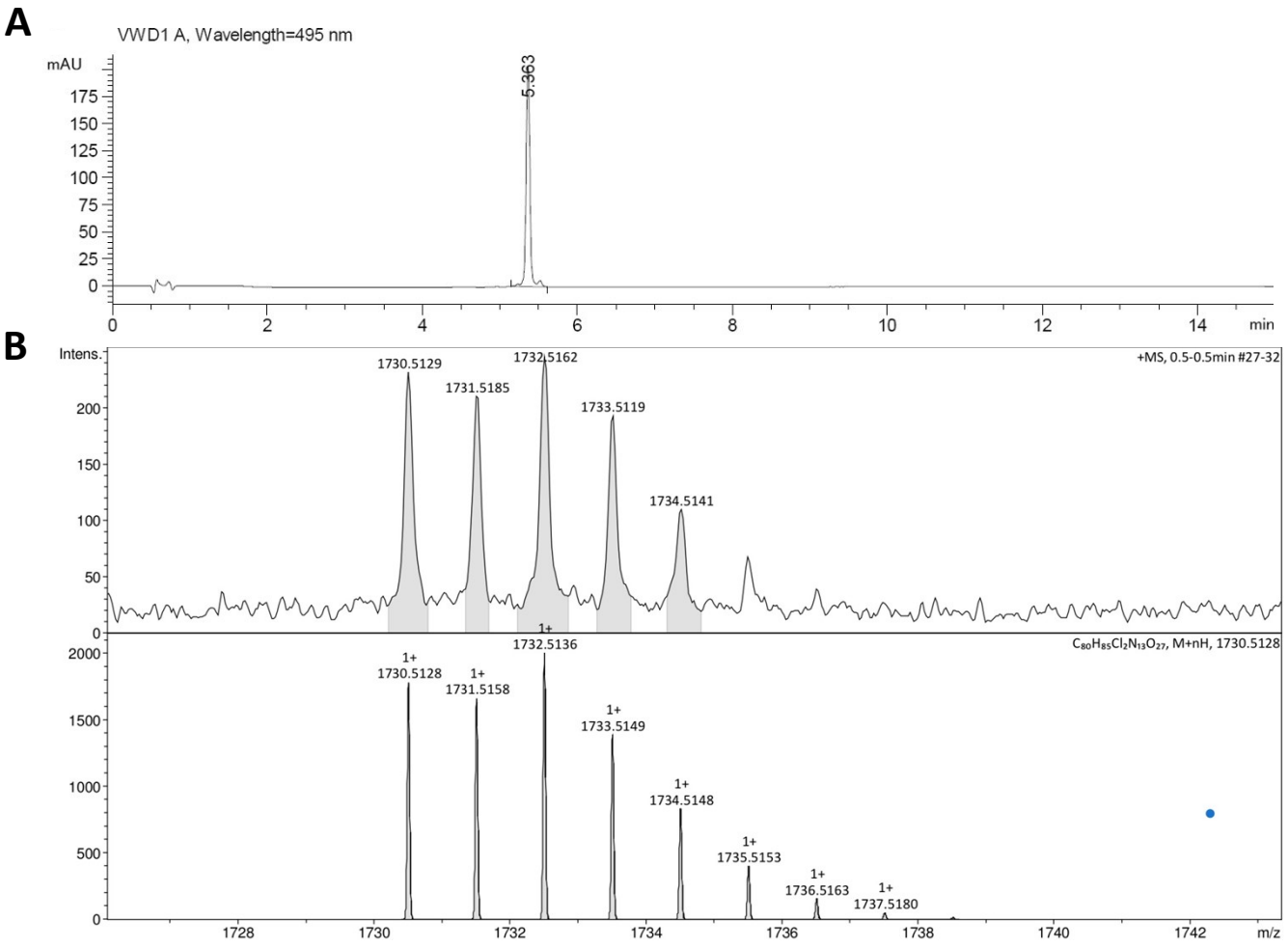


Figure S4. **A)** HPLC trace of ***Van-Green***. **B)** HRMS spectra of ***Van-Green***

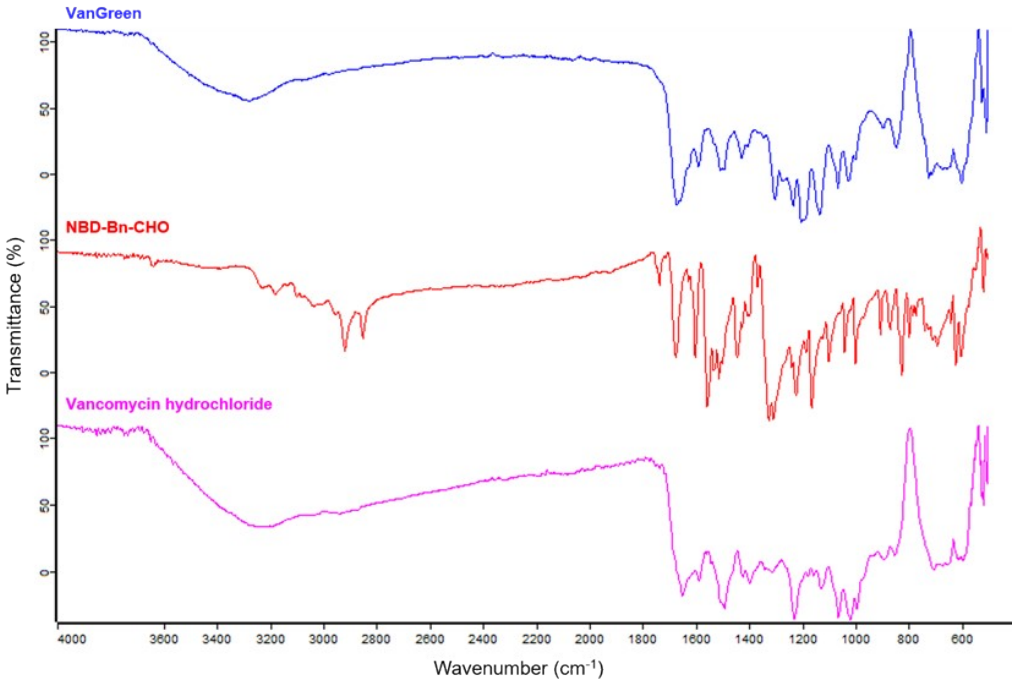


Figure S5. Stacked FT-IR spectra of Vancomycin hydrochloride (bottom, purple), NBD-Bn-CHO (middle, red) and of the final probe VanGreen (top, blue).

2. Optical characterisation

Photophysical studies of the Van-Green probe in solution were performed with freshly prepared air-equilibrated solutions at room temperature (298 K). UV/Vis absorption spectra were recorded on an Agilent 8453 spectrophotometer. Steady-state fluorescence measurements were performed on dilute solutions (optical density ≤ 0.1) using a Shimadzu RF-6000 spectrofluorometer. The emission spectra were corrected for the wavelength-sensitivity of the detection unit, obtained, for each compound, under excitation at the wavelength of the absorption maximum. Fluorescence quantum yields of these dilute chromophore solutions were measured according to literature procedures[1,2] using Fluorescein (FLSCN, Φ_f = 0.9 in NaOH 0.1 M, λ_{exc} = 474 nm) as reference.[3] The emission quantum yield values derived from these measurements were calculated with the following equation taking into account the refractive index n, the absorbance A, and the integral of the emission $I_f(\lambda_{exc},\lambda_f)$ of the novel probe (superscript *S*) relative to the reference (superscript ref):

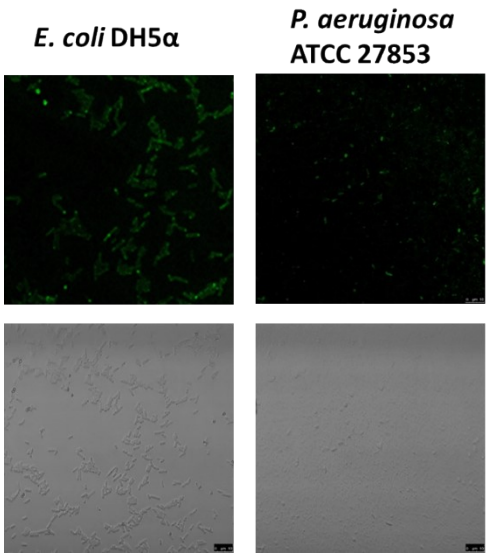
$$\Phi_f^S = \Phi_f^{ref} \times \left(\frac{n^S}{n^{ref}}\right)^2 \times \frac{1 - 10^{-A^{ref}(\lambda_{exc})}}{1 - 10^{-A^S(\lambda_{exc})}} \times \frac{\int_0^\infty I_f^S(\lambda_{exc},\lambda_f)\, d\lambda_f}{\int_0^\infty I_f^{ref}(\lambda_{exc},\lambda_f)\, d\lambda_f}$$

Refractive index values for all water/DMSO mixtures investigated here were taken from literature.[4]

Table S1: Photophysical properties of the **Van-Green** probe in different mixtures of PBS/DMSO.

PBS/DMSO mixture (*v*/*v*)	λ_{abs}^{max} (nm)	ε^{max} (M^{-1} cm^{-1})	λ_{em}^{max} (nm)	Stokes Shift (cm^{-1})	Φ_f[a]	$\varepsilon^{max}\Phi_f$ (M^{-1} cm^{-1})
1/0	480	1.2×10^4	535	2142	0.05	6.0×10^2
9/1	480	2.2×10^4	538	2246	0.07	1.6×10^3
8/2	478	2.3×10^4	542	2470	0.09	2.1×10^3
6/4	478	2.1×10^4	548	2672	0.16	3.3×10^3
4/6	475	1.9×10^4	546	2738	0.27	5.0×10^3
2/8	477	2.3×10^4	542	2514	0.36	8.2×10^3
0/1	480	2.0×10^4	539	2280	0.68	1.4×10^4

[a] Fluorescence quantum yield measured upon excitation at the maximum of absorption band, relative to Fluorescein in NaOH 0.1 M (Φ_f = 0.90).



3. Biological validation

Figure S6. Left: Negligible labelling of *E.coli DH5α, P. aeruginosa* ATCC 27853 by Van-Green 1 µM (top panel: fluorescence; bottom panel: brightfield).

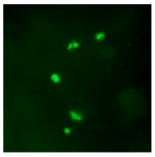
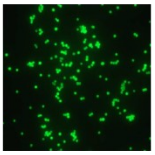
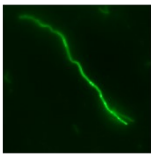
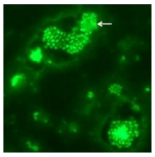
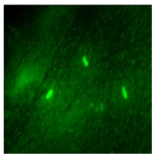
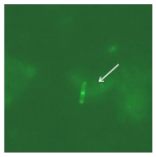
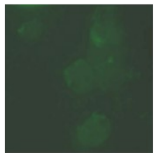
Fluorescence imaging with Bac3 probe							
Specimen	Pus from eye lid	Eviscerated material	Canalicular pus	Corneal Scraping	Corneal Scraping	Pus from orbit	Corneal scraping
Culture	Staphylococcus aureus	Staphylococcus aureus	Actinomycetes spp	Streptococcus pneumoniae	Corynebacterium spp	Bacillus cereus	Pseudomonas aeruginosa
Results of Gram staining	Few Gram positive cocci in singles, pairs and clusters	Plenty of Gram positive cocci in pairs, chains and clusters	Plenty of Gram positive filamentous bacilli morphologically resembling Actinomycetes/ Nocardia	Plenty of Gram positive cocci in pairs	Gram positive bacilli seen	Occasional stout Gram positive bacilli seen	Gram negative bacilli seen

Figure S7: Validation of Van-Green probe in clinical samples. All the samples were also analysed by Gram staining for comparison.

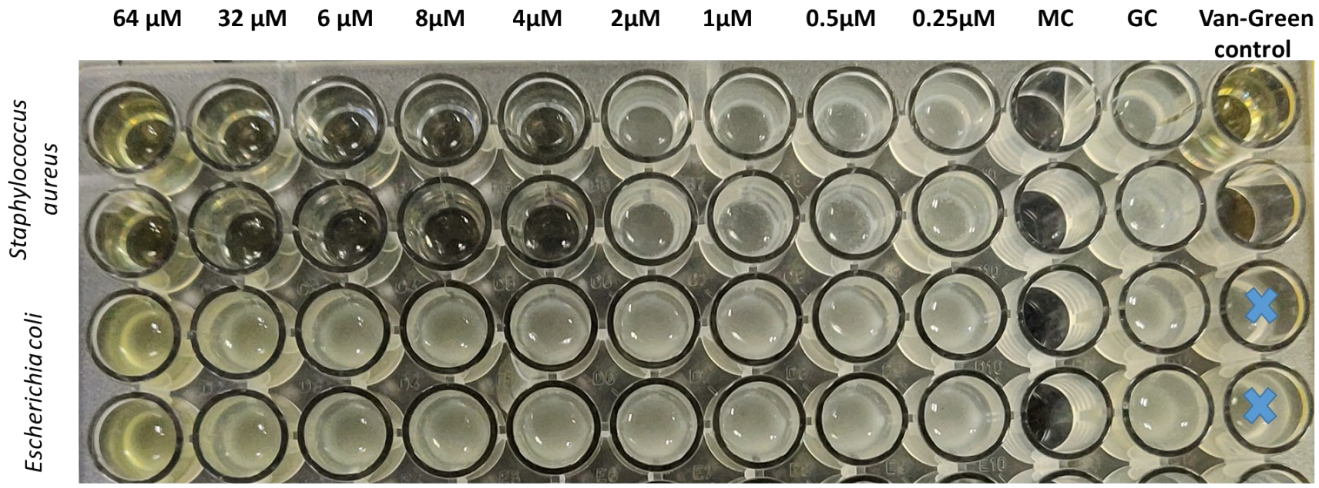
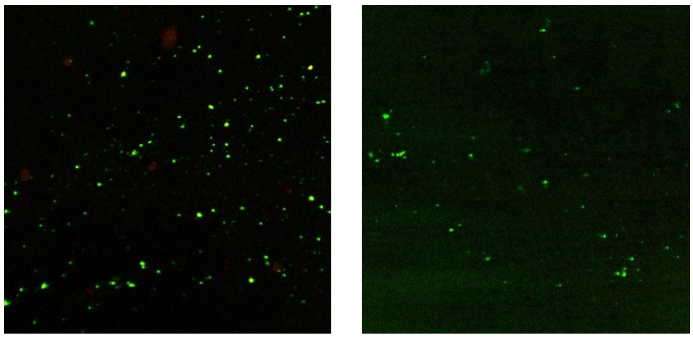


Figure S8: Minimum inhibitory concentration (MIC) experiment performed in a 96 well-plate on *S. aureus* and *E. coli* incubated with the probe **Van-Green** at various concentrations.



Van-Green **Vancomycin**

Figure S9: Live-dead staining of Gram positive cocci incubated with Van-Green and Vancomycin. Images are composite of green and red emission channels.

4. References

1 C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nat. Protoc.*, 2013, **8**, 1535–1550.
2 U. Resch-Genger and K. Rurack, *Pure Appl. Chem.*, 2013, **85**, 2005–2013.
3 A. M. Brouwer, *Pure Appl. Chem.*, 2011, **83**, 2213–2228.
4 R. G. LeBel and D. A. I. Goring, *J. Chem. Eng. Data*, 1962, **7**, 100–101.