Supporting Information for the Article

Evaluating the Potential of Vancomycin-modified Magnetic Beads as a Tool for Sample Preparation in Diagnostic Assays

Susanne Pahlow[1,2,3], Sabine Schmidt[3], Tabea Pappert[3], Lara Thieme[4], Oliwia Makarewicz[4], Stefan Monecke[1,2,3], Ralf Ehricht[1,2,3], Karina Weber[1,2,3] and Jürgen Popp[1,2,3]

1) Friedrich Schiller University Jena, Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, 07743 Jena, Germany
2) InfectoGnostics Research Campus Jena, Center for Applied Research, Philosophenweg 7, 07743 Jena, Germany
3) Leibniz Institute of Photonic Technology, Albert-Einstein-Straße 9, 07745 Jena, Germany, Member of the research alliance "Leibniz Health Technologies" and the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany
4) Institute of Infectious Diseases and Infection Control, University Hospital Jena, Erlanger Allee 103, 07747 Jena, Germany, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany

Table S1. Overview of selected publications investigating vancomycin modified particles and surfaces for the isolation of bacteria.

Reference	Particle/Surface Type	Particle Size	Functional Group Particle	Immobilization Method	Binding Site Vancomycin	Investigated Species (Yield)	CFU/mL	GP Specific	Application	Quantification /Detection Method
Abafogi et al. (1)	magnetic nanoparticles coated with polydopamine	100 nm	-Phenyl	Covalent binding between primary amine of vancomycin with phenyl rings of PDA	$-NH_2$	*S. aureus* MRSA *B. cereus* PBS: 80% Blood: 90%	$10 – 10^4$ CFU/mL	n/d	Isolation and preconcentration of bacteria from blood to enhance the sensitivity of PCR-based detection schemes	CFU counting
Azzam et al. (2)	Fe_3O_4 nanoparticles	56 nm	$-NH_2$	Conjugation of amine groups of MNPs with poly-L-Ornithine, then coupling of PEG-modified vancomycin	-COOH	*B. cereus* *P. aeruginosa* *E. coli* *K. pneumoniae*	$10 – 10^4$ CFU/mL	yes	Specific isolation of GP bacteria from water	CFU counting
Choi et al. (3)	Fe_3O_4 particles with photosensitizer [5,15-bisphenyl-10,20-bis(4-methoxycarbonylphenyl)-porphyrin] platinum	400 nm	n/a	Fe–carboxylate complex formation	-COOH	*S. aureus* (83%) MSSA (84%) MRSA (CI) (84 %) *E. faecalis* (88%) *B. cereus* (86%) VRE (CI) (85 %) *E. coli* (48 %) *S. typhimurium* (49 %)	10^6	GP: 85% GN: 48%	photodynamic inactivation of bacteria using magnetic particles with vancomycin and photosensitizer	CFU counting
Chung et al. (4)	Magnetofluorescent nanoparticles with $(Fe_2O_3)_m(Fe_3O_4)_n$ core conjugated with fluorescein and tetrazine	21 nm	Tetrazine	bioorthogonal cycloaddition between transcyclooctene modified Vanc and Tetrazine-modified magnetic particles (one-step vs. two-step approach)	$-NH_2$	*S. aureus* *S. pneumoniae* *S. epidermidis* *E. faecalis* *E. coli* *P. aeruginosa* *K. pneumoniae*	n/a	yes	Tagging Gram-positive bacteria both with magnetic and fluorescent label	Fluorescence intensity
Gu et al. (5)	FePt nanoparticles	3-4 nm	n/a	Pt–S and Fe–S Bonds between PtFe particles and bis(vancomycin) cystamide	n/a	*E. coli* White Blood Cells	$<10^2$	no	Separation of *E. coli* and white blood cells	Optical microscopy, SEM, TEM
Gu et al. (6)	FePt nanoparticles	3-4 nm	n/a	Pt–S and Fe–S Bonds between PtFe particles and bis(vancomycin) cystamide	n/a	*S. aureus* *S. epidermidis* coagulase negative staphylococci white blood cells	$<10^2$	n/d	Enrichment of Gram-positive and negative bacteria	Optical microscopy, SEM, TEM
Gu et al. (7)	Au nanoparticles (AuNPs)	3-4 nm	n/a	Au–S Bonds between Au	n/a	*E. faecium* *E. faecalis*	n/d	no	Enhanced antimicrobial	TEM

				particles and bis(vancomycin) cystamide		*E. coli*			activity of vancomycin by coupling to AuNPs	
Huang et al. (8)	Pt nanoparticles	n/a	COOH		$-NH_2$	*S. aureus* (92 – 110 %) *E. coli* *P. aeruginosa*	n/d	yes	Colorimetric detection of Gram-positive bacteria from liquid samples, i.e. fruit juice	Colorimetric and CFU counting
Hsu et al. (9)	Magnetic beads	180 nm	-COOH	Carbodiimide coupling with NHS	$-NH_2$	*E. coli* (19%) *P. aeruginosa* (48%) *K. pneumoniae* (11%) *E. faecalis* (48%) Clinical strains	10^6	no	Preconcentration for antimicrobial susceptibility testing	CFU counting (supernatant)
Kell et al. (10)	Iron oxide nanoparticles with Amine or carboxylic acid functionalized silica shell and commercially available magnetic beads	50 nm 1 µm 2.8 µm	$-NH_2$ -COOH	Carbodiimide coupling w/ or w/o NHS	NH_2 -COOH	*E. coli* *E. faecalis* *S. epidermidis* *P. aeruginosa* *S. agalactiae* *S. aureus* *K. oxytoca* *S. maltophilia* *E. faecium* (VRE)	30 - 300	Dependent on immobilization method	Systematic investigation of relation between particle size, coupling chemistry on isolation efficiency	CFU counting (supernatant)
Li et al. (11)	Magnetic beads (commercial)	180 nm	-COOH	Carbodiimide coupling with NHSS: 1) functionalization with Poly-L-Lysin linker to COOH 2) coupling of Vanc to PEG linker	-COOH	*B. cereus* (90 – 100%)	$10^1 – 10^6$	n/d	Isolation of *B. cereus* from milk with Magnetic beads with subsequent detection via PCR	n/a
Lin et al. (12)	Fe_3O_4 magnetic nanoparticles	11 nm	-OH	Carbodiimide coupling	-COOH	*S. aureus* *S. saprophyticus* *E. faecalis* *E. coli*	$10^4 - 10^8$	yes (preferred binding of GP)	Sample preparation for MALDI-MS, isolation from urine samples	OD_{600}
Meng et al. (13)	Carboxylated magnetic beads	180 nm	-COOH	Carbodiimide coupling with NHSS: 1) functionalization of MB with BSA, 2) coupling of Vanc to BSA	-COOH	*S. aureus* *L. monocytogenes* *B. cereus* *C. sakazakii* *E. coli* *S. enteritidis*	$10^4 – 10^5$	yes	Fluorescence: detection of captures *S. aureus* cells using a FITC-labelled antibody	CFU counting (supernatant)
Meng et al. (14)	Carboxylated magnetic beads	180 nm	-COOH	Carbodiimide coupling with NHSS: 1) functionalization with PEG linker to COOH 2)	-COOH	*E. coli* *S. typhimurium* *C. skazakii* *L. monocytogenes*	$10^4 – 10^6$	yes	Selective enrichment of GP bacteria (*L. monocytogenes*)	CFU counting (supernatant)

				coupling of Vanc to PEG linker					for PCR based detection	
Qi et al. (15)	mesoporous silica nanoparticles		$-NH_2$	Carbodiimide coupling with NHSS	-COOH	*E. coli* *S. aureus*		yes	Selective killing and fluorescence-based tracking of GP bacteria	Confocal laser scanning microscopy
Su et al. (16)	Magnetic beads	2 µm	-COOH	Carbodiimide coupling with NHSS	$-NH_2$	*S. mutans* *S. aureus* *B. cereus* *M. luteus* *E. coli* *S. dysenteriae* *S. typhimurium* *P. aeruginosa*	$10^2 – 10^7$	yes	Bioluminescence assay for quantification of viable Gram-positive bacteria	CFU counting (supernatant)
Shen et al. (17)	Magnetic beads (commercial)	200 nm	$-NH_2$	Carbodiimide coupling with NHSS	-COOH	*S. aureus (*90-91%) *S. hominis* (83-90%) *S. epidermidis* (50-51%) *E. gallinarium* (32-67%) The lower yields were obtained in urine, while the higher yields were achieved in PBST	10^7	n/d	Isolation of urinary tract pathogens with subsequent detection via MALDI ToF MS	Optical density measurements at 450 nm (OD_{450})
Wang et al. (18)	Magnetic beads	180 nm	-COOH	Carbodiimide (EDC) coupling with NHSS: 1) functionalization with PEG linker to COOH 2) coupling of Vanc to PEG linker	-COOH	*S. aureus* (90 % in PBS) MRSA (80 % in blood and cerebrospinal fluid)	$10^1 – 10^6$	n/d	Detection of MRSA in blood and cerebrospinal samples via PCR	CFU counting
Wang et al. (19)	Magnetic beads	180 nm	-COOH	Carbodiimide (EDC) coupling with NHSS: 1) functionalization with BSA 2) coupling of Vanc EDC/NHSS activated Vanc	-COOH	*S. aureus*	$3.3 \times 10^2 – 3.3 \times 10^8$	n/d	Detection of *S. aureus* in fruit juice via rolling circle amplification	n/s
Xue et al. (20)	Magnetic beads (commercial)	300 nm	-COOH	Carbodiimide (EDC) coupling with NHSS: Co-modification with Vancomycin and butyrylcholinesterase	$-NH_2$	*S. aureus* (80 – 100%)	$5 – 10^7$	yes	Detection of *S. aureus* using an immunoassay based on recognition of *S. aureus* via vancomycin and Protein A/IgG interaction in	CFU counting

									drinking water, fruit juice, milk and pork leachate	

Abbreviations: CFU – Colony forming units, EDC - 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, GN – Gram-negative, GP – Gram-positive, MALDI ToF MS - matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MRSA - Methicillin-resistant *Staphylococcus aureus*, MSSA - Methicillin-sensitive *Staphylococcus aureus*, NHS - N-Hydroxysuccinimide, NHSS - N-Hydroxysulfosuccinimide, OD_{600} – Optical density measured at 600 nm, PBS – phosphate buffered saline, PDA – Polydopamine, PEG – Polyethylene glycol, SEM - scanning electron microscopy, TEM - transmission electron microscopy, VRE – Vancomycin-resistant Enterococci, VSE - Vancomycin-sensitive Enterococci

Table S2. List of bacterial strains applied within this study.

Species	Strain	Van resistance	Temperature	Medium
Escherichia coli	ATCC 25922		37 °C	LB, LA
Escherichia coli	ATCC 35218		37 °C	LB, LA
Escherichia coli	DSM 10806		37 °C	LB, LA
Escherichia coli	UK013		37 °C	LB, LA
Escherichia coli	UK014		37 °C	LB, LA
Acinetobacter baumannii	UK011		30 °C	LB, LA
Acinetobacter baumannii	UK012		30 °C	LB, LA
Acinetobacter baumannii	DSM 30007		30 °C	LB, LA
Acinetobacter baumannii	UK20		30 °C	LB, LA
Acinetobacter baumannii	UK21		30 °C	LB, LA
Acinetobacter baumannii	UK22		30 °C	LB, LA
Klebsiella pneumoniae	DSM 30104		37 °C	LB, LA
Klebsiella pneumoniae	UK26		37 °C	LB, LA
Klebsiella pneumoniae	UK27		37 °C	LB, LA
Klebsiella pneumoniae	UK28		37 °C	LB, LA
Staphylococcus aureus	UK001		37 °C	LB, LA
Staphylococcus aureus	UK002		37 °C	LB, LA
Staphylococcus aureus	DSM 20231		37 °C	LB, LA
Staphylococcus aureus	ATCC 29213		37 °C	LB, LA
Staphylococcus aureus	UK17		37 °C	LB, LA
Staphylococcus aureus	UK18		37 °C	LB, LA
Staphylococcus aureus	UK19		37 °C	LB, LA
Staphylococcus cohnii	DSM 6719		37 °C	LB, LA
Staphylococcus cohnii	DSM 6718		37 °C	LB, LA
Staphylococcus cohnii	DSM 20261		37 °C	LB, LA
Staphylococcus cohnii	DSM 20262		37 °C	LB, LA
Staphylococcus warneri	DSM 20036		37 °C	LB, LA
Staphylococcus warneri	DSM 20316		37 °C	LB, LA
Enterococcus faecium	DSM 20477		37 °C	TSY, TA
Enterococcus faecium	UK005		37 °C	TSY, TA
Enterococcus faecium	UK006	VRE	37 °C	TSY, TA
Enterococcus faecium	UK035	VSE, TS	37 °C	TSY, TA
Enterococcus faecium	UK036	VSE, TS	37 °C	TSY, TA
Enterococcus faecium	UK037	VSE, TS	37 °C	TSY, TA
Enterococcus faecium	UK038	VSE, TS	37 °C	TSY, TA
Enterococcus faecium	UK039	VSE, TS	37 °C	TSY, TA
Enterococcus faecium	UK040	vanA, TR	37 °C	TSY, TA
Enterococcus faecium	UK041	vanA, TR	37 °C	TSY, TA
Enterococcus faecium	UK042	vanA, TR	37 °C	TSY, TA
Enterococcus faecium	UK043	vanB, TS	37 °C	TSY, TA
Enterococcus faecium	UK044	vanB, TS	37 °C	TSY, TA
Enterococcus faecalis	UK003		37 °C	TSY, BA

Enterococcus faecalis	UK004	37 °C	TSY, BA
Enterococcus faecalis	UK045	37 °C	TSY, BA
Enterococcus faecalis	UK046	37 °C	TSY, BA
Enterococcus faecalis	UK047	37 °C	TSY, BA
Enterococcus faecalis	UK048	37 °C	TSY, BA
Enterococcus faecalis	UK049	37 °C	TSY, BA

LB = Lysogeny Broth, TSY = Trypticase Soy Yeast Extract Medium, BA = Blood Agar Plates, TA = TSY Agar Plates

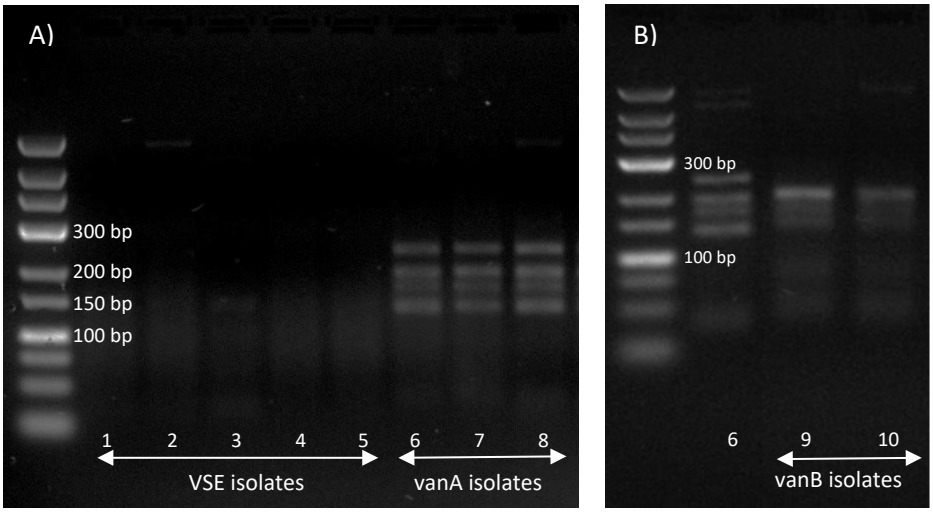


Figure S1. Restriction fragment length patterns of the *E. faecium* isolates. PCR products of vanA, vanB and vanC-1 genes were digested with MspI and separated by gel electrophoresis. Expected sizes (bp) of MspI restriction fragments were the following: vanA 231, 184, 163, 131/133, vanB 188/189, 160, 136; vanC-1 230/237 (21). **A)** 1 = UK035, 2 = UK036, 3 = UK037, 4 = UK038, 5 = UK039, 6 = UK040, 7 = UK041, 8 = UK042, **B)** 9 = UK043, 10 = UK044.

Table S3. Phenotypic and genotypic characterization and clinical data of the *E. faecium* isolates.

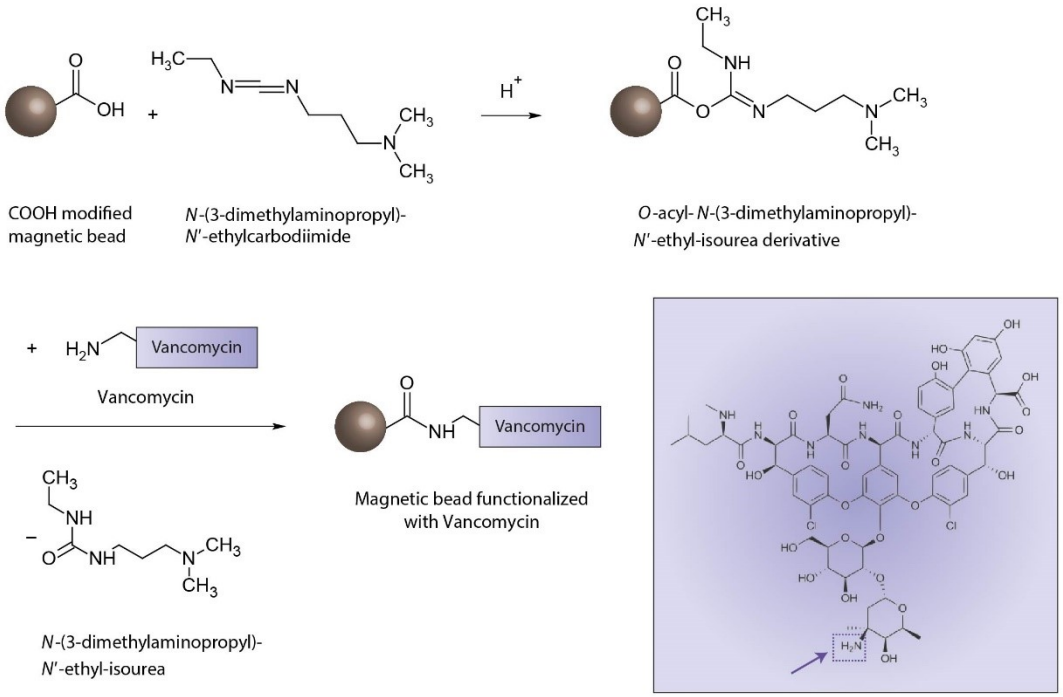
Isolate	Vancomycin susceptibility	Teicoplanin susceptibility	Van Resistance type	Clinical background	Sex	Age (years)
UK035	S	S	/	recurrent bacteraemia	f	63
UK036	S	S	/	endocarditis	m	77
UK037	S	S	/	urosepsis, catheter-related	f	86
UK038	S	S	/	endocarditis	m	76
UK039	S	S	/	endocarditis	m	62
UK040	R	R	vanA	recurrent bacteraemia, nosocomial	m	54
UK041	R	R	vanA	spinal disc infection	f	74
UK042	R	R	vanA	bacteraemia	f	49
UK043	R	S	vanB	endocarditis	m	69
UK044	R	S	vanB	colonisation	f	62

R = resistant. S = susceptible. Vancomycin and teicoplanin susceptibility was determined by Vitek (bioMerieux, Marcy-l'Etoile, France).

Table S4. Clinical data of the *E. faecalis* isolates.

Isolate	Clinical background	Sex	Age (years)
UK045	urosepsis	m	76
UK046	endocarditis	m	75
UK047	opportunistic infection after liver transplant rejection	m	60
UK048	opportunistic infection without clinical signs	f	55
UK049	urosepsis, catheter-related	f	67

a) Functionalization of COOH Magnetic Beads with Vancomycin using EDC



b) Functionalization of COOH Magnetic Beads with Vancomycin using EDC and NHSS

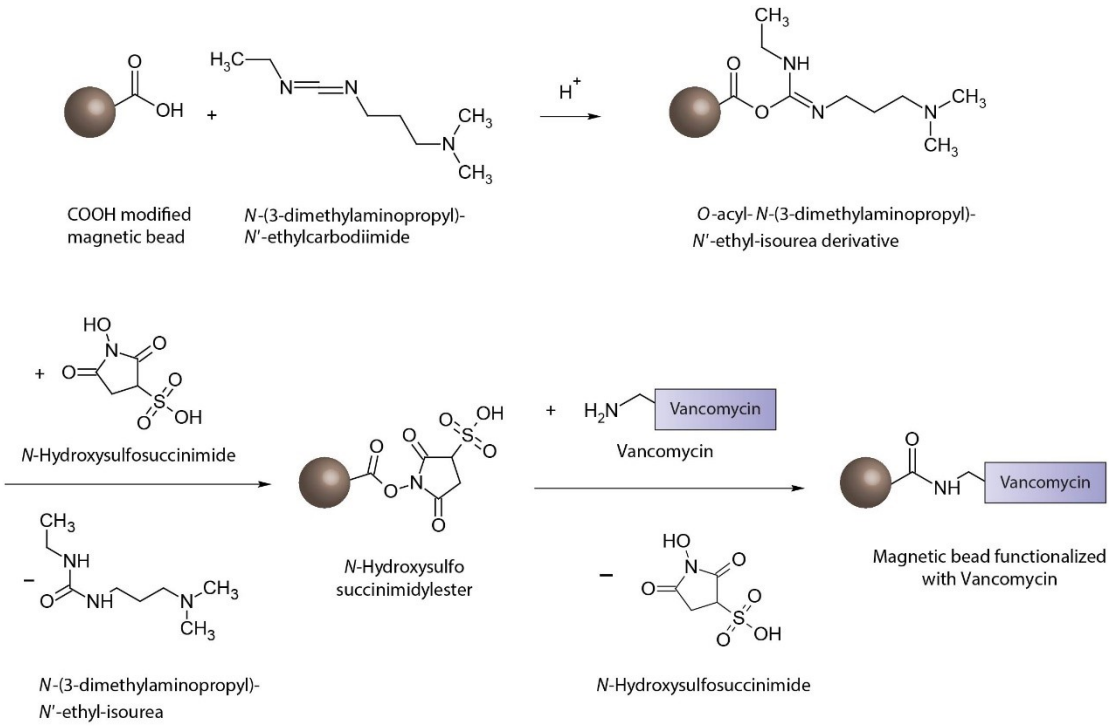


Figure S2. Reaction schemes for functionalization of carboxylic acid modified magnetic beads with vancomycin a) using EDC or b) EDC and NHSS.

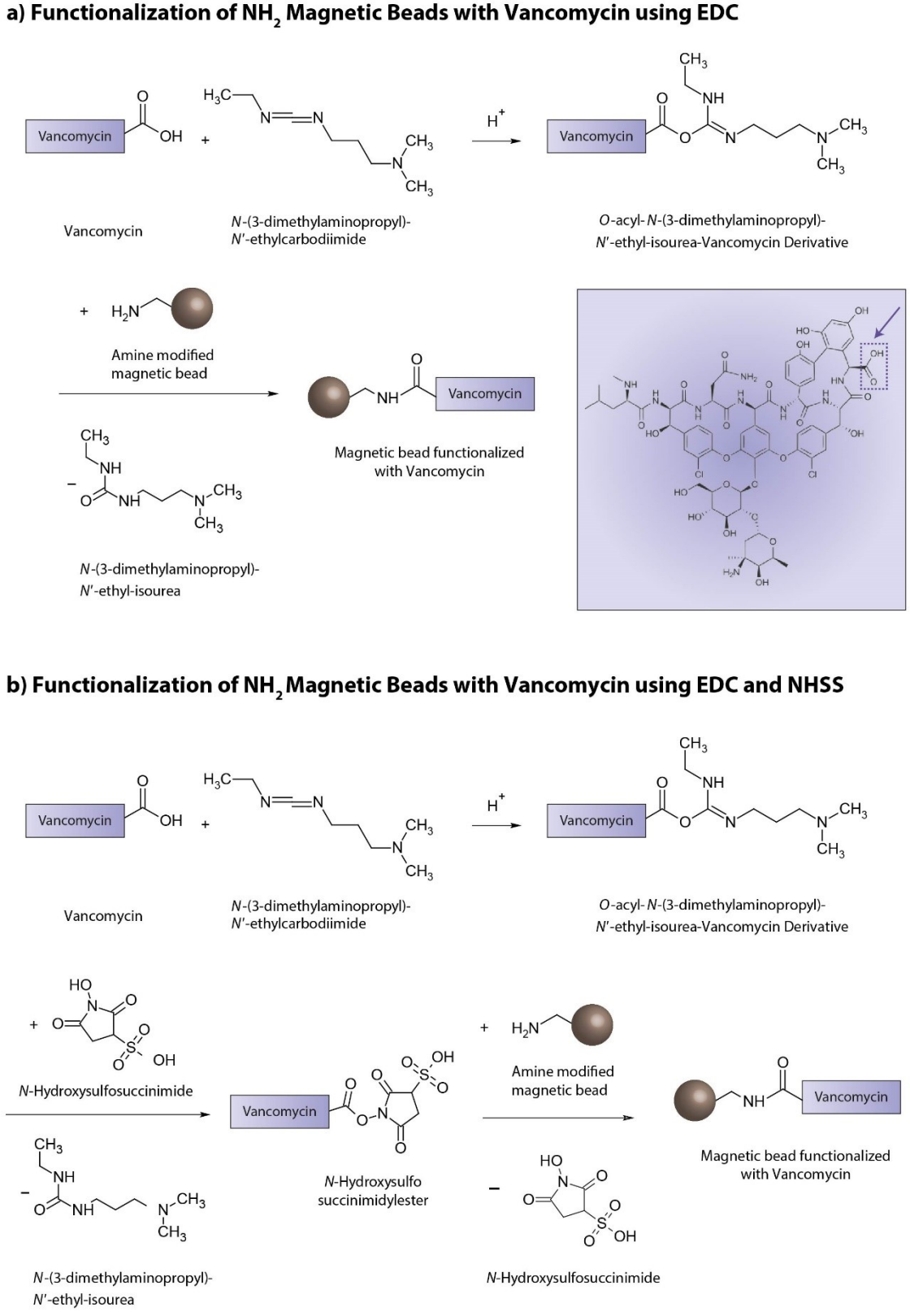


Figure S3. Reaction schemes for functionalization of amine modified magnetic beads with vancomycin a) using EDC or b) EDC and NHSS.

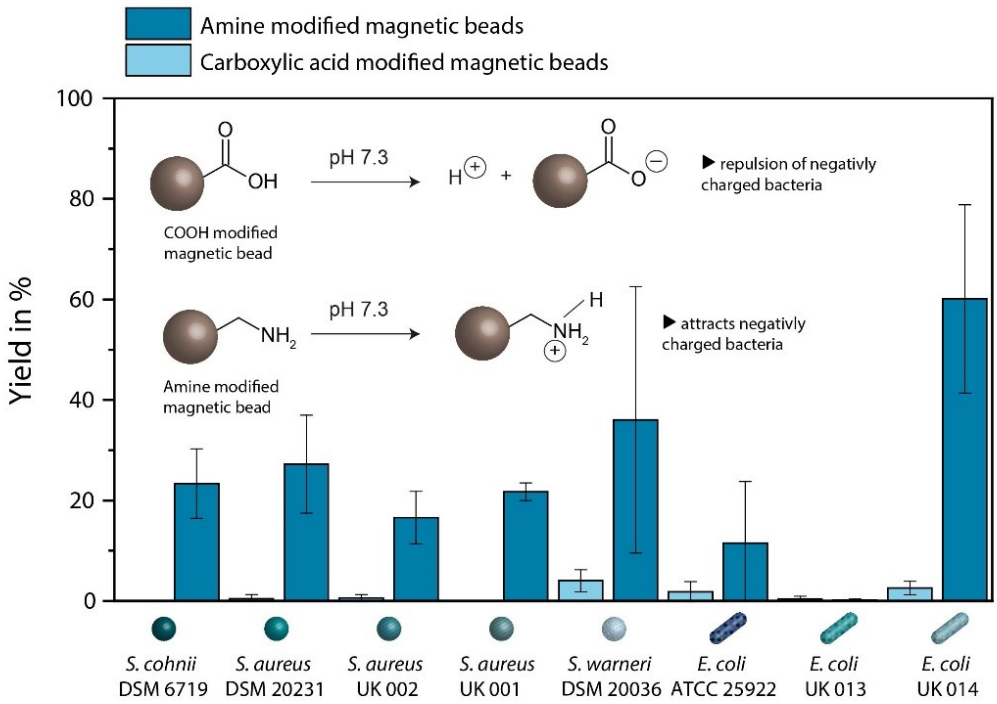
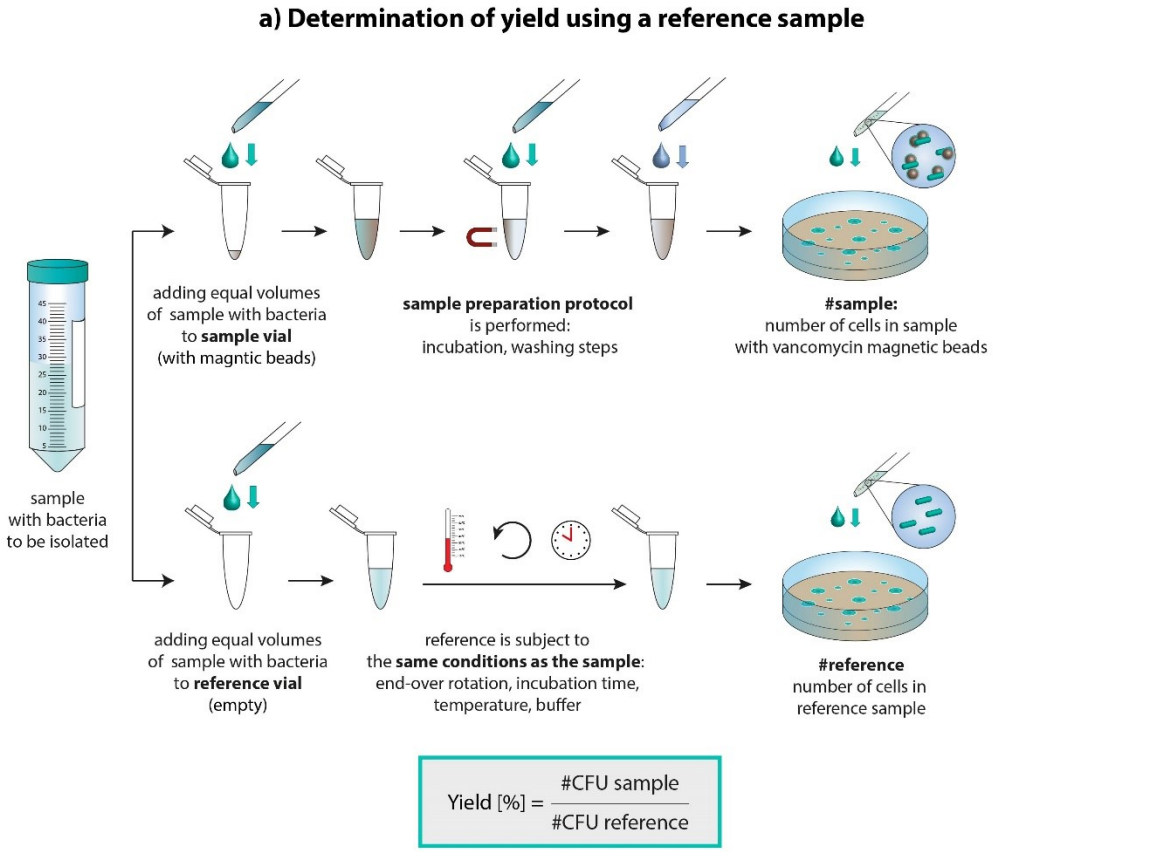


Figure S4. Comparison of isolation yields for dynabeads M-270 Carboxylic acid and M-270 Amine after incubating them with different bacterial species in 1x Phosphate buffered saline (1x PBS) for 1 h, followed by three washing steps with 1x PBS. Yields were determined using plate counting.



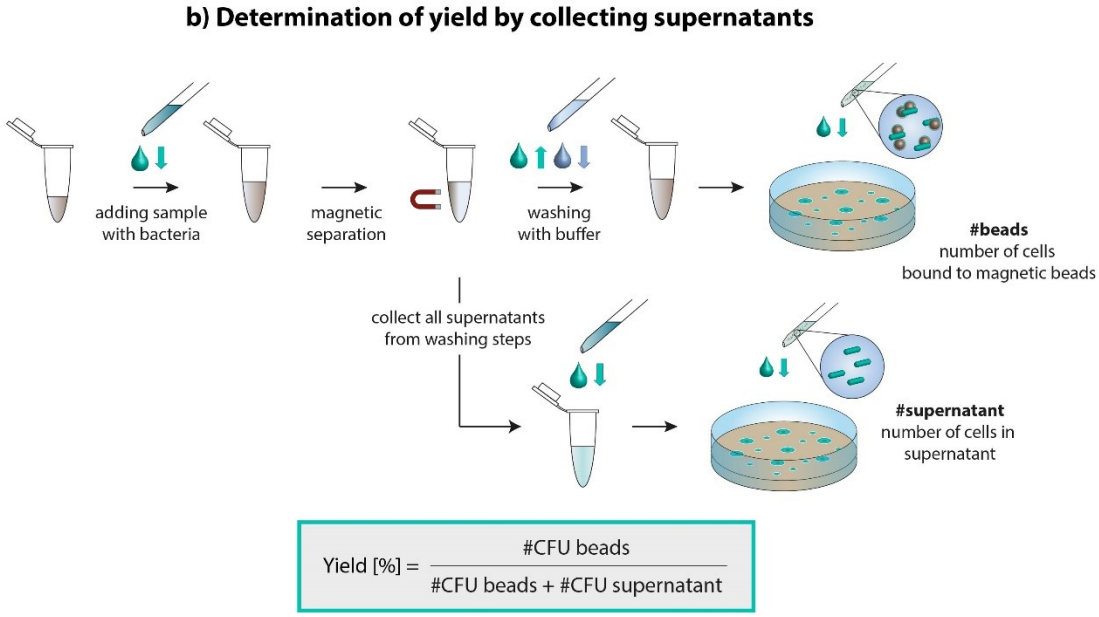


Figure S5. Examples for methods to determine the yield in magnetic bead-based sample preparation schemes: a) via reference sample b) by collecting supernatants.

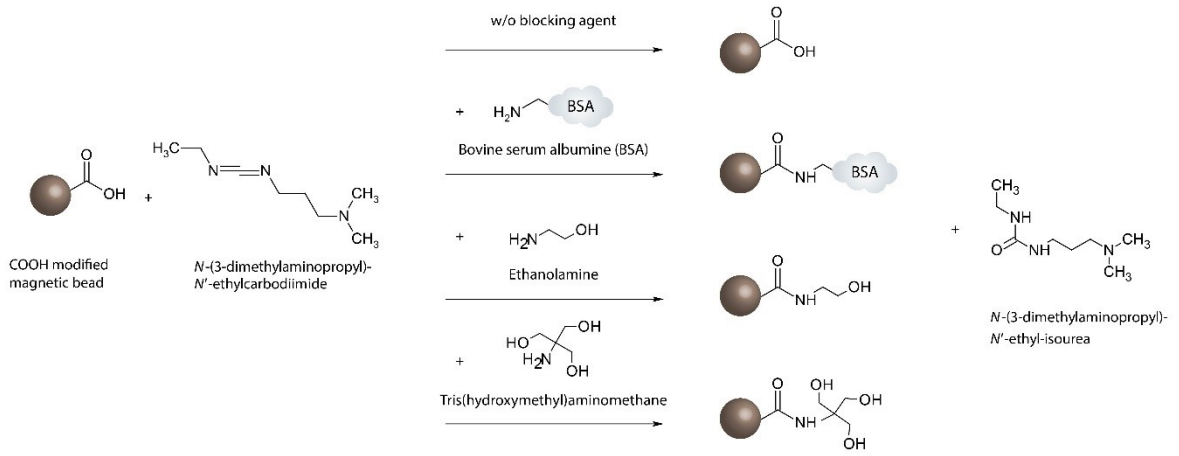


Figure S6. Reaction scheme for functionalizing carboxylic acid magnetic beads with different blocking agents.

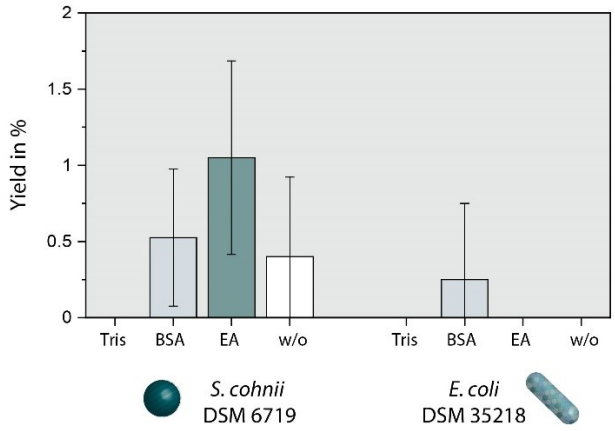


Figure S7. Comparison of affinity of magnetic beads functionalized with different blocking agents (Tris: Tris(hydroxymethyl)aminomethane, BSA: bovine serum albumin, EA: ethanolamine, w/o: without) to *S. cohnii* and *E. coli* cells.

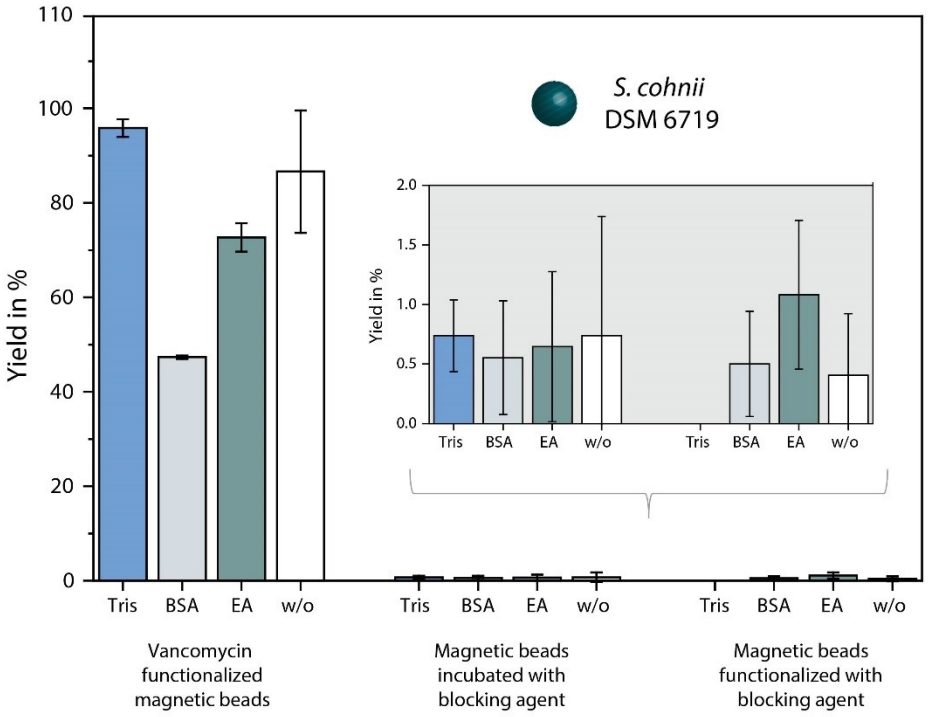


Figure S8. Influence of different blocking agents (Tris: Tris(hydroxymethyl)aminomethane, BSA: bovine serum albumin, EA: ethanolamine, w/o: without) on isolation efficiency for vancomycin beads, carboxylic acid magnetic beads incubated or functionalized with blocking agent for *S. cohnii*.

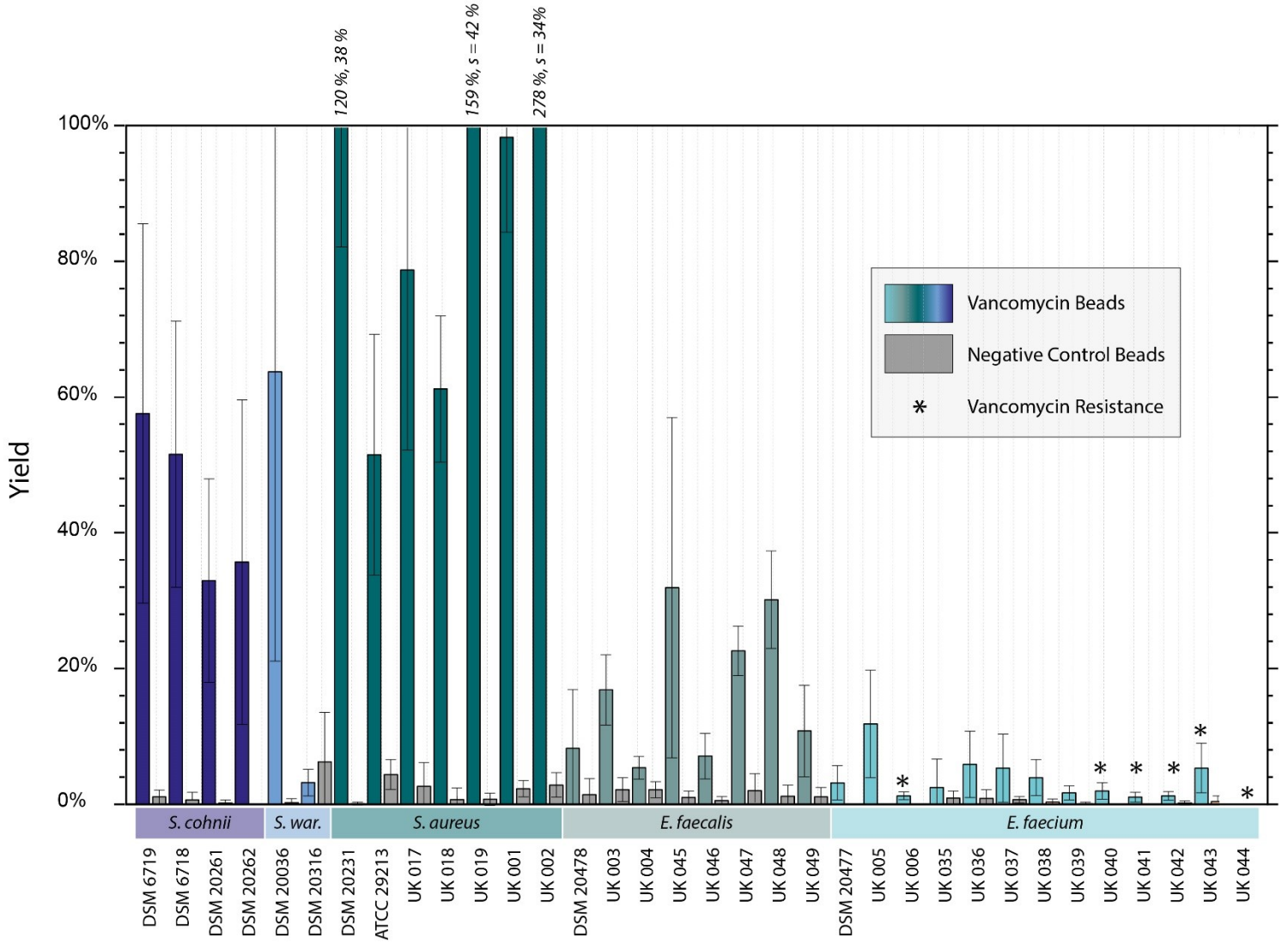


Figure S9. Isolation yields for vancomycin functionalized and control beads for different Gram-positive species and strains.

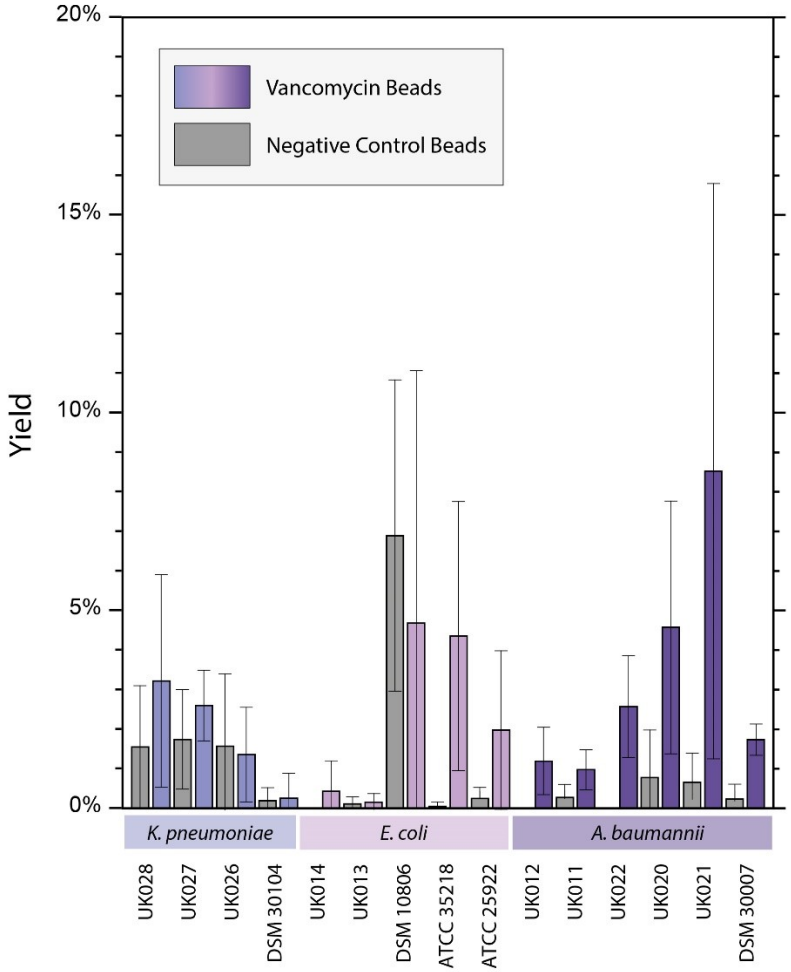


Figure S10. Isolation yields for vancomycin functionalized and control beads for different Gram-negative species and strains.

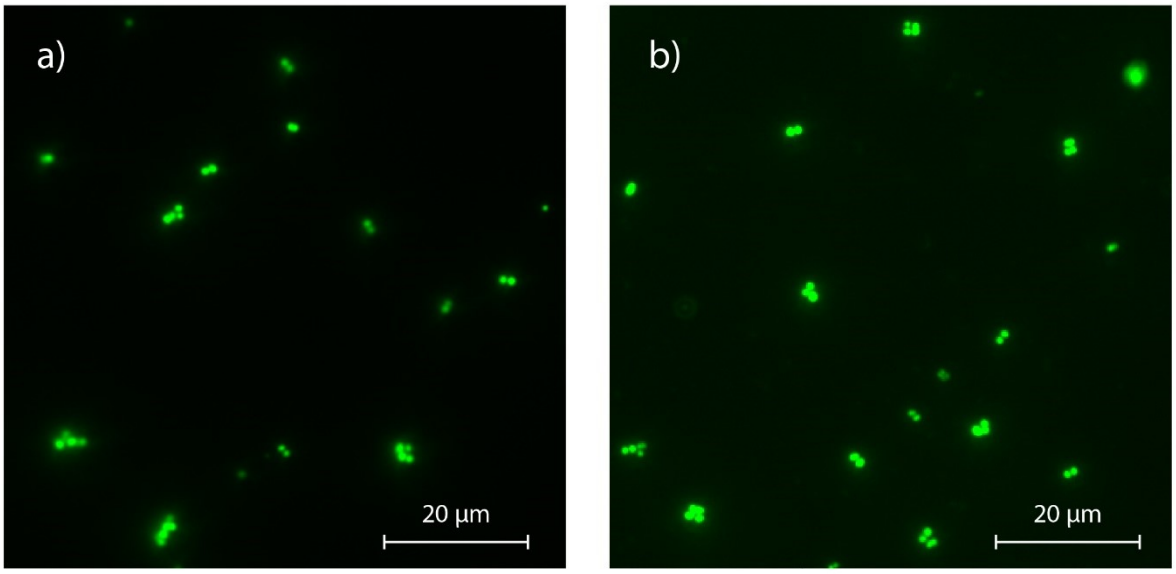


Figure S11. Fluorescence microscopic images of *S. aureus* cells stained with Syto 9. a) *S. aureus* DSM 20231, b) *S. aureus* UK019. The cells were grown in liquid culture until the early exponential phase and directly stained within the medium without further treatment or washing steps.

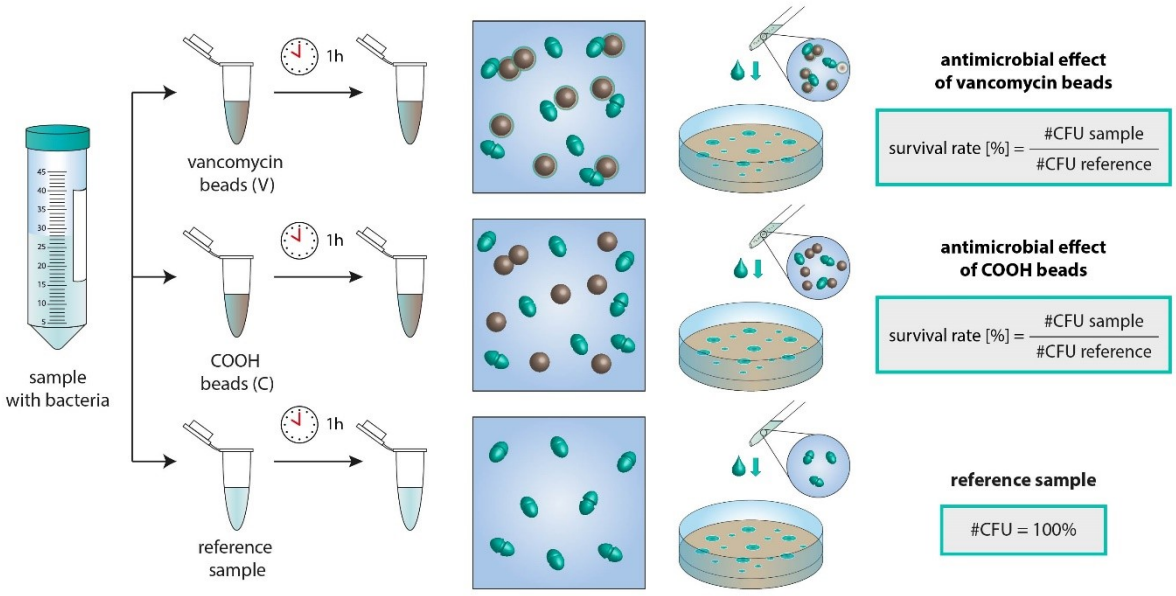


Figure S12. Experimental scheme for evaluating the antimicrobial effect of vancomycin beads.

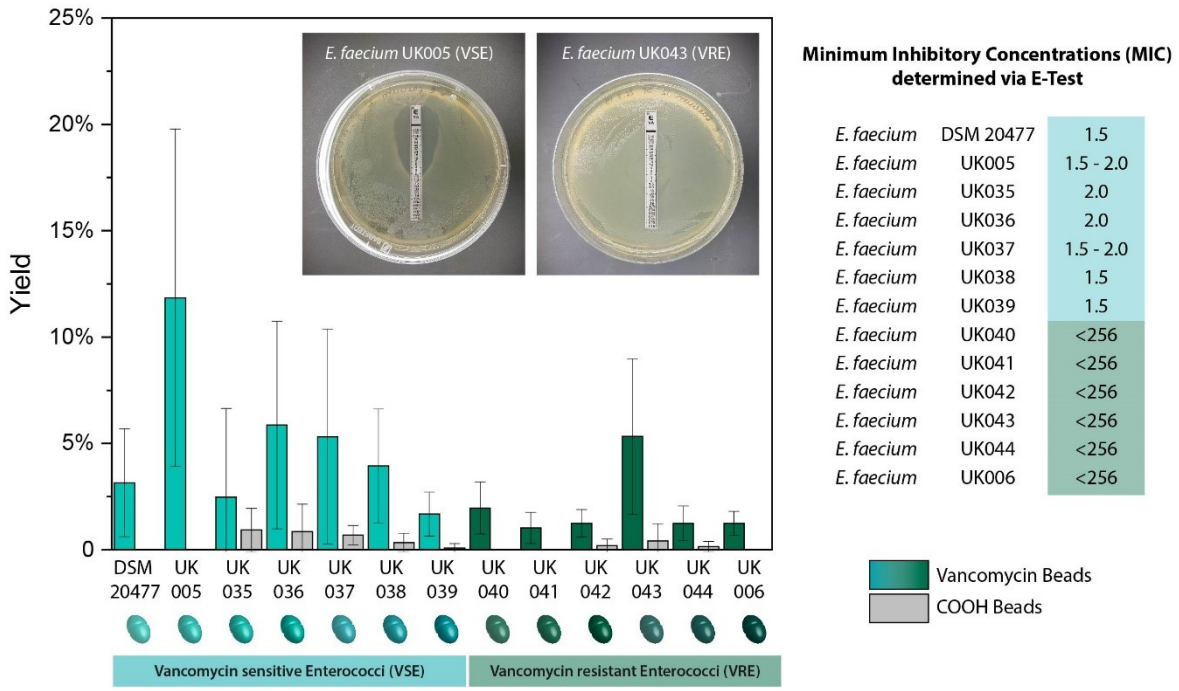


Figure S13. Yields for *E. faecium* strains ordered by vancomycin resistance achieved with vancomycin functionalized beads. Vancomycin resistance or sensitivity was verified using using an epsilometer test (E-Test).

Practical Guide for Establishing a Vancomycin Bead-based Isolation Protocol for Bacterial Cells

Establishing a sample preparation protocol is a complex task and requires many decisions and a considerable amount of time. Based on our experience we compiled some important hints and questions to enable you to design your experiments in a more efficient manner and hopefully avoid unnecessary frustration.

Choosing a quantification method

When deciding how to evaluate your results, the determining factor should be your intended application or detection method. For example, if you want to perform further experiments such as antibiotic susceptibility testing demanding viable cells or apply Raman microspectroscopy on intact single cells, colony forming unit (CFU) counting is a sensible choice as it allows you to directly judge how many cells meeting your requirements can be retrieved with the isolation protocol. On the other hand, when it is irrelevant to you whether the cells are viable or not, but you are more interested in the total cells number, then a nucleic acid-based method such as quantitative PCR (qPCR) or an immunoassay are better suited for you. No matter what you choose, it is important to be aware of the limitations, so you can interpret your results properly and draw correct conclusions.

PCR as quantification method

It will be relevant, whether you perform the lysis with the cells still bound to the magnetic beads or after a detachment step. In the first case, it is possible that the DNA binds to the bead surface due to electrostatic interactions, so the yields appear reduced. In that instance it can be worthwhile to optimize the pH and ionic strength of the buffer, so that unwanted binding of nucleic acids to the bead surface is minimized. When the cells are released from the magnetic beads before performing the lysis step, it can be advisable to investigate how many cells remain on the beads. Depending on the antimicrobial effect of the beads, either CFU counting or microscopic images can aid in getting an idea of the efficiency of the detachment step.

CFU counting as quantification method

The main advantage of determining yields via CFU counting lies in its simplicity. However, the experiments need to be well designed in order to retrieve the desired information. It goes without saying, that the method is only suitable, when it can be ensured that the vast majority of the cells in the desired sample type will be viable. In the following we list some aspects to consider, when planning your experiments:

> ***Do the species have a known tendency to form clusters or other agglomerates?*** For example, Staphylococci form clusters, while Streptococci often grow in chains (*S. pyogenes*) or duplicates (*S. pneumoniae*). Bacilli also can form long chains or are found in pairs. Such properties can significantly influence the CFU number, especially when mixing the bacterial cells with the beads breaks up the agglomerates. Acquiring microscopic images from the cultured cells, will provide this information. While vigorous mixing is not enough to separate the cells, sonication can be a solution. Haaber et al. developed a simple and efficient protocol based on sonication allowing for the correct enumeration of *S. aureus.* (22) If for some reason separation of cells cannot be achieved, it might be helpful to add vancomycin beads to the reference sample. By doing so, the cells in the reference sample will be subject to the same degree of disruption as

the cells in the sample for determining the isolation yield. Accordingly, a more realistic estimation of the yield can be achieved.

How to design the reference sample? There are different options for treating the reference sample. Again, the concrete choice depends on what information will be most relevant to you. The basic idea is to determine how efficiently you can isolate cells from a defined sample. (A) From an application-based perspective the best way is to plate small volumes directly from the same bacterial sample that is added to the magnetic beads without any delay. Positive or negative effects on bacterial growth due to the treatment steps (incubation, shaking, antimicrobial effect of beads etc.) will be reflected in the calculated yield. As these steps are part of the sample preparation they should be accounted for when specifying the yield. A deviation from this proceeding can however be advised, when the determination of the correct cell number of the initial sample is hampered by a tendency of the cells to form clusters or other arrangements. Depending on your possibilities either a sonication treatment, determination of the cell concentration via qPCR or adding equal amounts of vancomycin beads to the reference sample and incubating as long as the sample, with which the isolation is performed, are possibilities. (B) When you are mainly interested in the effect of the capture molecule, we recommend including both a reference sample that contains the equal volume and concentration of bacterial cells as the vancomycin bead sample and a control sample identical to the vancomycin bead sample except that instead of vancomycin beads non-functionalized beads are included. (C) Moreover, it is possible to estimate the yield without a dedicated reference sample by collecting the supernatants of the samples and plating these as well. The initial number of cells in the sample is determined by adding the numbers of the isolated cells and those remaining in the supernatant. This is a practical solution with some advantages. Similar to the previously described approach positive or adverse effects of the vancomycin beads on cells are not reflected in the yield. On the other side, the results can be distorted, when the vancomycin beads have significant antimicrobial effect. Then it is hard to draw valid conclusions on the initial number of cells present in the sample. (D) In case the formation of large agglomerates between bacterial cells and vancomycin beads is very pronounced, direct plating of the beads after isolation will probably not result in the correct number of isolated cells. In this case we recommend plating a sample just with the bacterial cells for determining the initial concentration and collecting the supernatants from the washing steps to deduce the number of cells remaining on the beads indirectly.

Specificity Gram-positive vs. Gram-negative

Theoretically, vancomycin-functionalized beads should specifically bind Gram-positive bacteria and only vancomycin sensitive strains. In practice, however, deviations from this behavior can occur. It seems the bead material and functional groups on the beads, the immobilization method, blocking buffer and also the pH value of the sample during incubation with the magnetic beads are all factors influencing the specificity of the vancomycin-bacterial cell interaction. Depending on your planned application either performance can be advantageous. You might want to enrich a broad spectrum of Gram-positive and Gram-negative species or only targeting vancomycin sensitive species might be critical for the specificity of your whole assay. We recommend choosing a protocol from literature that displays the desired outcome and using it as a starting point for optimization. Since cell membrane damage can lead to exposure of the peptidoglycan layer in Gram-negative bacteria, it makes sense to take into account what pretreatment the bacterial cells will experience before the vancomycin bead

isolation procedure is carried out. It can be helpful to gather information on the isoelectric points of the bacterial species and other components involved to get an idea of electrostatic forces involved.

Antimicrobial effect of vancomycin beads

When using CFU counting for evaluating the capture efficiency obviously the antimicrobial effect of the vancomycin beads has to be considered. The intensity of the effect will be affected by the bead size, as it determines how many interactions between bacterial cell wall and the antibiotic can occur. There are two basic options for testing the antimicrobial activity of the vancomycin beads. Agar diffusion tests can visualize to what extent vancomycin is released from the particles, while incubating a cell sample with vancomycin functionalized beads will display the combined effect of the antibiotic and the mechanical stress from mixing the beads with the cells. Our data suggests that the antimicrobial effect can differ from strain to strain and of course between species.

Magnetic Beads

A wide range of magnetic beads with different sizes, materials and surface modifications is commercially available. Furthermore, it is also a possibility to synthesize them yourself with customized properties, in case this is within your field of expertise. It is impossible to make a specific recommendation in terms of immobilization chemistry. Our advice is to use the well-designed study of Kell et al. as orientation. (10) They systematically investigated the effect of the vancomycin binding site and also tested the influence of linker molecules. Additionally, we refer you to the overview of vancomycin bead studies provided in **Table 1** in the main article and choose the one that is the best fit in terms of specificity and range of species as starting point. Another important factor is the ratio of beads to bacterial cells. In most cases it is advisable to use an excess of beads to obtain the highest possible yields.

Negative controls

Negative controls can be defined in different ways. Commonly, a negative control refers to a sample which does not contain the target organism or analyte and will therefore yield a negative test result. In the context of developing a sample preparation protocol, which is only part of a diagnostic assay, the definition differs. Within our study we implemented two types of negative controls: 1) samples without bacteria in order to check for contamination and 2) non-functionalized magnetic beads to verify the successful surface functionalization and to investigate the efficiency of the capture molecule. We recommend including both types as each one provides important information.

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