Gold nanoparticle smartphone platform for diagnosing urinary tract infections

Supporting Information

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**Table S1:** List of genetic sequences used in the study.

| NAME | GENETIC SEQUENCE | DESCRIPTION |
|------|------------------|-------------|
| **FIGURE 2A** | | |
| SRT.-E.coli-V1* | rGrGrArArGrGrArArGrUrArUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr UrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr UrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr UrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr UrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr UrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUr Ur

**FIGURES 2B/C, S3 and S4**

| NAME | GENETIC SEQUENCE | DESCRIPTION |
|------|------------------|-------------|
| Mz.-E.coli-V2/R | GCCGCTGCTGCGACGAGTTAACAGACAGAGAACACCT | Right half of Mz. binds E. coli 16S rRNA region 2, can bind and cleave Linker-E.coli |
| Mz.-E.coli-V2/L | TGCCCCAGGGAGCTAGCTAAGCGGTGCTTTCTCTGGGG | Left half of Mz. binds E. coli 16S rRNA region 2, can bind and cleave Linker-E.coli |
| Linker | AGCATGTGTGATAGGGTGTTTCTCTCC(TG)(U) | Crosslinks DNA_L and DNA_R, can be cleaved by Mz.-E.coli-V2 |
| DNA_L | HS--AAAAAATTGACTAGCATGCT | Adsorbs onto GNP through thiol, hybridized to Linker |
| DNA_R | GCCGTAGCTGTTTAAGAAAAAAA--SH | Adsorbs onto GNP through thiol, hybridized to Linker |

**FIGURES 3 and S8**

| NAME | GENETIC SEQUENCE | DESCRIPTION |
|------|------------------|-------------|
| E. coli | | |
| Linker-E.coli | CAGATGCTAGCTAAGCGGTGCTTTCTCTGGGG | Crosslinks DNA_L, E.coli and DNA_R, E.coli, can be cleaved by Mz.-E.coli |
| DNA_L-E.coli | HS--AAAAAATTGACTAGCATGCT | Adsorbs onto GNP through thiol, hybridized to Linker-E.coli |
| DNA_R-E.coli | GCCGTAGCTGTTTAAGAAAAAAA--SH | Adsorbs onto GNP through thiol, hybridized to Linker-E.coli |
| S. saprophyticus | | |
| Linker-Staph | GCCCTACTGCAACCCGTTATCCGACACACACACGCAGCTTGAAGATGGGGG | Crosslinks DNA_L, S. saprophyticus and DNA_R, S. saprophyticus, can be cleaved by Mz.-Staph |
| DNA_L-Staph | HS--AAAAAATTGACTAGCATGCT | Adsorbs onto GNP through thiol, hybridized to Linker-Staph |
| DNA_R-Staph | GCCGTAGCTGTTTAAGAAAAAAA--SH | Adsorbs onto GNP through thiol, hybridized to Linker-Staph |
| P. mirabilis | | |
| Linker-Prot | GAAGATGCTGTTTAAGAAAAAAA--SH | Crosslinks DNA_L, P. mirabilis and DNA_R, P. mirabilis, can be cleaved by Mz.-Prot |
| DNA_L-Prot | HS--AAAAAATTGACTAGCATGCT | Adsorbs onto GNP through thiol, hybridized to Linker-Prot |
| DNA_R-Prot | GCCGTAGCTGTTTAAGAAAAAAA--SH | Adsorbs onto GNP through thiol, hybridized to Linker-Prot |
### E. faecalis

**Target region**

TCTTTCTCCGCCAGTGTGATCATTGCAAAGAGAGGAGT

Region of 16S rRNA gene highly conserved within *E. faecalis* species, targeted by Mz-Faec.

**Mz-Faec/L**

AATCTATACGCTGCAACGCTCGGAGGAAG

Left half of Mz, binds *P. mirabilis* target region, can bind and cleave Linker-Faec.

**Mz-Faec/R**

GAGTAACACCCAATAATGTGGGACAGAAGGAAAGAGGAGT

Right half of Mz, binds *P. mirabilis* target region, can bind and cleave Linker-Faec.

**Linker-Faec**

GAGTAACACCCAATAATGTGGGACAGAAGGAAAGAGGAGT

Crosslinks DNA_L-Faec and DNA_R-Faec, can be cleaved by Mz-Faec.

**DNA, L-Faec**

HS-AAAAAATATTATTGCTTACT

Adsorbs onto GNP through thiol, hybridizes to Linker-Faec.

**DNA, R-Faec**

TGTAAAGGAGTAGTAATAAAAAA-AG-AAAAAAA-SH

Adsorbs onto GNP through thiol, hybridizes to Linker-Faec.

### K. pneumoniae

**Target region**

CATGCAAGTGGAGGAGGATGAGACAGAGGCTTGTCTC

Region of 16S rRNA gene highly conserved within *K. pneumoniae* and *E. aerogenes* species, targeted by Mz-Kleb.

**Mz-Kleb/R**

ACCCGAGCAAGCCTCTAGCAACAGTAGATTGGGTGAG

Right half of Mz, binds *K. pneumoniae* target region, can bind and cleave Linker-Kleb.

**Mz-Kleb/L**

CAGAGCTGTCTAGGCTACTGACTGCAGCTACITT

Left half of Mz, binds *K. pneumoniae* target region, can bind and cleave Linker-Kleb.

**Linker-Kleb**

ACATACATGCAGCATCTCGCCAAACATA(G)G

Left half of Mz, binds *UBP* Linker-Kleb, can bind and cleave Linker-Kleb.

**DNA, L-Kleb**

HS-AAAAAAATATTATTGCTTACT

Adsorbs onto GNP through thiol, hybridizes to Linker-Kleb.

**DNA, R-Kleb**

AGAGGAAATTAGTCATAAAAA-AG-AAAAAAA-SH

Adsorbs onto GNP through thiol, hybridizes to Linker-Kleb.

### UBP

**Target region**

AACAGCAGTATTAGACGTTCTCTGACAGCGCGGT

Region of 16S rRNA gene highly conserved in all UTI bacteria, targeted by Mz-UBP.

**Mz-UBP/L**

ACCCGAGCAAGCCTCTAGCAACAGTAGATTGGGTGAG

Right half of Mz, binds *UBP* target region, can bind and cleave Linker-UBP.

**Mz-UBP/R**

CAGAGCTGTCTAGGCTACTGACTGCAGCTACITT

Left half of Mz, binds *UBP* target region, can bind and cleave Linker-UBP.

**Linker-UBP**

ACATACATGCAGCATCTCGCCAAACATA(G)G

Crosslinks DNA_L-UBP and DNA_R-UBP, can be cleaved by Mz-UBP.

**DNA, L-UBP**

HS-AAAAAAATATTATTGCTTACT

Adsorbs onto GNP through thiol, hybridizes to Linker-UBP.

**DNA, R-UBP**

AGAGGAAATTAGTCATAAAAA-AG-AAAAAAA-SH

Adsorbs onto GNP through thiol, hybridizes to Linker-UBP.

### FIGURE S1

**SDT-Ecoli-V1**

GGAGGAGGAAGTAAAGGATTATACCTTGTGCTATGAGGTGAC

Synthetic DNA target equivalent to E. coli 16S rRNA region 1.

**SDT-Ecoli-V2**

CCCCGCGAGAAGAAGCAGCGCTACTCGCTGCAAGCGCC

Synthetic DNA target equivalent to E. coli 16S rRNA region 2.

**SDT-Ecoli-V3**

TTACGCGAGAAGAAGCAGCGCTACTCGCTGCAAGCGCC

Synthetic DNA target equivalent to E. coli 16S rRNA region 3.

**Mz-Ecoli-V1/R**

GGCCGTGCTGGACAGGATTACAAAGGAGGAGAAGGAGGAGT

Right half of Mz, binds E. coli 16S rRNA region 1, can bind and cleave Linker-Ecoli.

**Mz-Ecoli-V1/L**

TGGCCAGCGAGCTACTGCTCGGGGTGCTTTCTCTCTCTC

Left half of Mz, binds E. coli 16S rRNA region 1, can bind and cleave Linker-Ecoli.

**Mz-Ecoli-V2/R**

GGCCGTGCTGGACAGGATTACAAAGGAGGAGAAGGAGGAGT

Right half of Mz, binds E. coli 16S rRNA region 2, can bind and cleave Linker-Ecoli.

**Mz-Ecoli-V2/L**

TGGCCAGCGAGCTACTGCTCGGGGTGCTTTCTCTCTC

Left half of Mz, binds E. coli 16S rRNA region 2, can bind and cleave Linker-Ecoli.

**Mz-Ecoli-V3/R**

GGCCGTGCTGGACAGGATTACAAAGGAGGAGAAGGAGGAGT

Right half of Mz, binds E. coli 16S rRNA region 3, can bind and cleave Linker-Ecoli.

**Mz-Ecoli-V3/L**

TGGCCAGCGAGCTACTGCTCGGGGTGCTTTCTCTC

Left half of Mz, binds E. coli 16S rRNA region 3, can bind and cleave Linker-Ecoli.

**Linker**

AGCAATGGTCAAGCTGCAAGCTTCTGCATCTCGGCAG

Crosslinks DNA_L and DNA_R, can be cleaved by Mz-Ecoli-V2.

**DNA, L**

HS-AAAAAAATATTATTGCTTACT

Adsorbs onto GNP through thiol, hybridized to Linker.

**DNA, R**

GGAGGAGGAAGTAAAGGATTATACCTTGTGCTATGAGGTGAC

Adsorbs onto GNP through thiol, hybridizes to Linker.
**Figure S2**

| NAME             | GENETIC SEQUENCE                                      | DESCRIPTION                                                                 |
|------------------|--------------------------------------------------------|------------------------------------------------------------------------------|
| Mz-E.coli-V2/R   | GCGGCTGCTGGCAGGGAGTTCAACGAGAGGGAAACCTT                | Right half of Mz, binds E. coli 16S rRNA region 2, can bind and cleave Linker |
| Mz-E.coli-V2/L   | TGCCCAGGGAGCTAGCTAGCCGGTGCTTCTCTGCGGG                | Left half of Mz, binds E. coli 16S rRNA region 2, can bind and cleave Linker  |
| in1-Mz-E.coli-V2/L| TGCCCAGGGAAAGCTAGCTAGCCGGTGCTTCTTCTGCGGG             | Mz-E.coli-V2/L inactivated by a single base mutation (G → A) in the catalytic core |
| in2-Mz-E.coli-V2/L| TGCCCAGGGAGCTAGCTAGCCGGTGCTTCTGCGGG                | Mz-E.coli-V2/L inactivated by scrambling the catalytic core                   |
| ns-Mz-E.coli-V2/R | AGCTGCTGCCGTGGTAGACACGAGACAGAAACCTT                 | Right half of Mz, binds the same linker as Mz-E.coli-V2, but cannot be activated by E. coli RNA |
| ns-Mz-E.coli-V2/R | TGCCCAGGGAGCTAGCTCCATTGCCCCATGTAAGTCA               | Left half of Mz, binds the same linker Mz-E.coli-V2, but cannot be activated by E. coli RNA |
| Linker           | AGCTGCTGCCGTGGTAGACACGAGACAGAAACCTT                 | Crosslinks DNA_L and DNA_R, can be cleaved by Mz-E.coli-V2                   |
| DNA_L            | HS–AAAAAAACCTCTAGACATGCT                             | Adsorbs onto GNP through thiol, hybridized to Linker                          |
| DNA_R            | GCGCTAGCTGCTGTTAAAAAAAA–SH                           | Adsorbs onto GNP through thiol, hybridized to Linker                          |

* RNA bases are preceded by ‘r’
** Each Mz half includes 3 regions: (i) region that binds the target, indicated in bold, (ii) region that binds the Linker DNA, indicated in italic script, and (iii) one half of the catalytic core, underlined.
*** Each Linker strand contains two regions that bind DNA-A and DNA-B (indicated in bold), and a region that is bound by the Mz (underlined), includes 2 RNA bases in the middle.
**** Each DNA_L and DNA_R contain a thiol group (indicated in bold) for attachment for GNP, poly-A spacer (italicized), and the region bound by the Linker (underlined).

**Table S2: Iterative development of E. coli specific Mz**

| Iteration | Mz             | Accepted | Reason for Rejection                                      |
|-----------|----------------|----------|-----------------------------------------------------------|
| 1         | Mz-E.coli-v1   | NO       | Low sensitivity due to poor GNP aggregation                |
| 2         | Mz-E.coli-v2   | NO       | Cross-reactivity with other bacteria                        |
| 3         | Mz-E.coli-v3   | NO       | Cross-reactivity with other bacteria                        |
| 4         | Mz-E.coli-v4   | NO       | Low sensitivity due to poor Mz activation                  |
| 5         | Mz-E.coli-v5   | NO       | Low sensitivity due to poor Mz activation                  |
| 6         | Mz-E.coli-v6   | NO       | Cross-reactivity with other bacteria                        |
| 7         | Mz-E.coli-v7   | YES      |                                                            |
**Table S3:** Cost estimates for the Mz-GNP smartphone platform.

| REAGENT                                      | UNITS         | COST PER UNIT  | NET COST |
|----------------------------------------------|---------------|----------------|----------|
| **RNA Extraction (cost per sample)**         |               |                |          |
| 1. Commercial Kit (Biobasic)                 |               |                |          |
| 1 RNA Extraction (column + buffers)          | 1 extraction  | $1.26/extraction | $1.26   |
| TOTAL                                        |               |                | $1.26    |
| 2. In-house Kit                              |               |                |          |
| Silica Column                                | 1 column      | $0.16/column   | $0.16    |
| Buffers                                      | 3 mL          | $20.00/liter   | $0.06    |
| TOTAL                                        |               |                | $0.22    |
| **Mz-GNP Assay (cost per sample for all 6 parallel UTI panel Mz reactions)** |               |                |          |
| DNA-Functionalized Gold Nanoparticles         | 3.3x10^-13 moles | $4.57x10^9/mole | $0.01   |
| Linker and Mz DNA Components                 | 1.5x10^-11 moles | $5.69x10^9/mole | $0.09   |
| TLC Plate                                    | 1/10th of a plate | $0.60/plate     | $0.06   |
| Buffers                                      | 0.01 mL       | $5.00/liter    | <<$0.01 |
| TOTAL                                        |               |                | $0.16    |
| **3D Printed Smartphone Readout System**     |               |                |          |
| LED Lights                                   | 4 pieces      | $1.80          | $7.20    |
| Plastic                                      | 217.39 grams  | $0.07          | $15.22   |
| Mini Breadboard                              | 1 piece       | $3.15          | $3.15    |
| Resistors                                    | 5 pieces      | $0.02          | $0.10    |
| Wire                                         | 5 pieces      | $0.23          | $1.15    |
| Battery                                      | 1 piece       | $4.25          | $4.25    |
| Switch                                       | 1 piece       | $1.25          | $1.25    |
| TOTAL                                        |               |                | $32.32   |
Supplementary Discussion S1

We previously demonstrated that the Mz-GNP assay could detect multiple oligonucleotide targets in parallel (1). The presence of non-specific sequences neither interfered with the detection nor gave false positives. However, all of the targets tested were from unrelated species and had minimal sequence similarity. In contrast, many UTI pathogens are closely related and have low sequence divergence in their 16S rRNA. Furthermore, our sequence analysis showed that some regions could be more divergent within the same species or genus than between different bacteria. Therefore, the careful selection process was followed to choose regions that were well conserved within the same species but divergent between the other species. 16S rRNA sequences were acquired from Greengenes (2) and StrainInfo (3) online databases, and online multiple sequence alignment with hierarchical clustering tool was used to locate the optimal target regions (4). For example, we found that the region targeted by the Mz Mz-E.coli-V2 could not be used for the panel since it was too strongly conserved and would lead to false-positive activation by Klebsiella, Proteus, and Enterobacter bacteria (Suppl. Figure S5). Another complexity arose because some regions of the 16S rRNA molecule have strong secondary structures, such as hairpins, which can inhibit the hybridization of the Mz target arms (5). Therefore, multiple regions had to be tested to identify the target that yielded the highest Mz catalytic activity. In general, the following scheme was followed to design each bacteria-specific Mz: (1) Align multiple 16S rRNA gene sequences for bacteria X. Locate several 41 bases long target regions that are highly conserved within bacteria X. (2) Align each of these target regions against multiple 16S rRNA sequences for bacteria Y. Discard the ones that have significant sequence similarity with bacteria Y. Repeat this for all other bacteria that might be present in the UTI samples, discarding the targets that show high sequence similarity with any bacteria except for X. (3) Design Mzs against the remaining target regions and test them against RNA extracted from bacteria X. Optimize the amount of the linker strand to determine the optimal concentration at which highest sensitivity can be achieved, but that causes visible GNP aggregation in the absence of the target (see ref. (1) for a full discussion on linker optimization). Discard the Mzs that yield sensitivity below 10^5 CFU/mL. (4) Confirm
the lack of cross-reactivity by verifying that RNA extracted from high concentrations of bacteria other than X does not activate the remaining Mzs. If none of the target regions satisfy the above criteria, restart with step (1) to find new target regions. The design of the UBP Mz that is activated by all of the bacteria was more straightforward since there are a number of 16S rRNA regions that are highly conserved among all bacterial species.

We followed the above recipe to generate Mzs targeting each bacteria in the UTI panel. For example, seven Mz variants had to be tested for *E. coli* before specific Mz that consistently yielded required detection sensitivity was identified (Suppl. Table S2). To carry out these iterations more efficiently, we simplified the protocol by reducing the number of RNA extraction steps. Instead of generating 10x dilution series of specific bacterial concentrations and then extracting RNA for each dilution, the total RNA was first extracted only for the highest dilution of $10^7$ CFU/mL and then serially diluted to correspond to the lower bacterial concentrations. Both methods were found to yield equivalent results (Figure S6).
Figure S1: (A) Testing multiple *E. coli* 16S rRNA Mzs with the RNA extracted from cultured bacteria to optimize GNP aggregation. (B) Comparing the sensitivity of Mz-*E.coli*-V2 and Mz-*E.coli*-V3 using synthetic DNA targets SDT_*E.coli*_V2 and SDT_*E.coli*_V3. ‘0’ represents negative control where water was used in place of the target. Of note, different Mzs produce negative spots of different colour (compare Mz-*E.coli*-V1 and Mz-*E.coli*-V1). We typically look at the difference in colour signal/intensity between the sample and control (0 CFU/mL) within the same experiment to make a diagnosis.
**Figure S2:** Mz control experiments for Figure 2 in the paper. Mz’s were tested with RNA extracted from *E. coli* spiked into LB Broth (left) or healthy urine (right) at $10^7$ bacteria/mL. Extractions using non-spiked LB culture medium and healthy urine were used for ‘0’ negative controls. MzV2 refers to Mz-*E.coli*-V2 in Table S1. ns-MzV2 – Mz can bind and cleave the same linker DNA as MzV2 but is activated by a different non-bacterial target. in1-MzV2 – MzV2 in which a key base required for catalysis was mutated. in2-MzV2 – MzV2 in which the catalytic core was scrambled.
Figure S3: Same as Figure 2B in the paper, but 5 mL instead of 1 mL of bacterial culture was used for RNA extraction. Higher volume results in an improvement in detection sensitivity to $10^4$ CFU/mL. Extractions using a non-spiked LB culture medium were used for ‘0’ negative controls.
| CFU / mL | 0  | 10^3 | 10^4 | 10^5 | 10^6 | 10^7 |
|----------|----|------|------|------|------|------|
| **Spin Column Kit** | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| **Magnetic Kit** | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| **Lysozyme + Proteinase K** | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |

**Figure S4:** Comparison of different RNA extraction methods. Spin Column Kit – the most common RNA extraction method that uses centrifugation-based RNA loading columns. Magnetic Kit – assay based on magnetic beads that adsorb RNA; centrifugation is replaced by magnetic extraction. Lysozyme + Proteinase K – these two enzymes lyse bacterial cell walls and proteins, and the mixture is used directly as the input sample for the Mz-GNP assay without any RNA purification steps. Extractions using non-spiked LB culture medium used for ‘0’ negative controls.
**Figure S5:** Alignment of the 16S rRNA of UTI panel bacteria around the region targeted by Mz\_E.coli\_V2. There is no sequence divergence between the *E. coli*, *K. pneumonia* and *P. mirabilis* bacteria. *Enterobacter* shows only a single base difference and will also activate the Mz\_E.coli\_V2.

| Bacterium                        | Mz\_E.coli\_V2_Target |
|----------------------------------|-----------------------|
| *E. coli*                        | Target                |
| *Klebsiella pneumonia*           | Target                |
| *Proteus mirabilis*              | Target                |
| *Enterobacter aerogenes*         | Target                |
| *Providencia stuartii*           | Target                |
| *Citrobacter freundii*           | Target                |
| *Escherichia coli*               | Target                |
| *Enterobacter cloacae*           | Target                |
| *Staphylococcus aureus*          | Target                |
| *Streptococcus agalactiae*       | Target                |

**Figure S6:** Comparing 2 methods of RNA extraction. (i) Cultured bacteria were first serial diluted from $10^7$ CFU/mL bacterial culture to indicated concentrations, then RNA extracted separately from each dilution. Extractions using a non-spiked LB culture medium were used for ‘0’ negative controls. (ii) RNA was first extracted from $10^7$ CFU/mL bacterial culture and then serially diluted in 10X increment to represent the different bacterial concentrations (e.g., 100X dilution would represent $10^5$ CFU/mL). Water used in place of extracted RNA for ‘0’ negative control.
**Figure S7:** Same as Figure 3 in the paper but including wavelengths corresponding to the peaks of absorbance spectra. Water used in place of extracted RNA for '0' negative control.
Escherichia coli

| E. coli | Proteus | Enterococcus | Staph | Klebsiella | UBP |
|--------|---------|--------------|-------|------------|-----|
| 106    |         |              |       |            |     |
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PROBES

PROBES
**Escherichia coli**

**Figure S8:** GNP-Mz UTI panel clinical results for samples from patients with *E. coli* infection. Each row represents a particular patient (identified by the patient number); each column indicates which GNP probe was used.
Figure S9: GNP-Mz UTI panel clinical results for samples from patients with *Proteus* infection. Each row represents a patient (identified by the patient number); each column indicates which GNP probe was used.
**Klebsiella pneumoniae**

|   | 128 | 129 | 130 | 131 | 140 | 141 | 142 | 143 | 157 | 158 | 159 | 160 | 161 | 162 | 163 | 164 | 165 |
|---|------|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| E. coli |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Proteus |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Enterococcus |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Staph |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Klebsiella |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| UBP |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**Figure S10:** GNP-Mz UTI panel clinical results for samples from patients with *Klebsiella* infection. Each row represents a patient (identified by the patient number); each column indicates which GNP probe was used.
**Escherichia coli, \( \leq 10^4 \text{ CFU/mL} \)**

|   | E. coli | Proteus | Enterococcus | Staph | Klebsiella | UBP |
|---|---------|---------|--------------|-------|------------|-----|
| 279 | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| 280 | ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) | ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |
| 281 | ![Image](image13.png) | ![Image](image14.png) | ![Image](image15.png) | ![Image](image16.png) | ![Image](image17.png) | ![Image](image18.png) |
| 282 | ![Image](image19.png) | ![Image](image20.png) | ![Image](image21.png) | ![Image](image22.png) | ![Image](image23.png) | ![Image](image24.png) |
| 283 | ![Image](image25.png) | ![Image](image26.png) | ![Image](image27.png) | ![Image](image28.png) | ![Image](image29.png) | ![Image](image30.png) |

**Figure S11:** GNP-Mz UTI panel clinical results for samples from patients with *E. coli* bacteria present at \( 10^4 \text{ CFU/mL} \) or below. Each row represents a patient (identified by the patient number); each column indicates which GNP probe was used.
Figure S12: GNP-Mz UTI panel clinical results for samples from *uninfected* patients. Each row represents a patient (identified by the patient number); each column indicates which GNP probe was used.
Figure S13: Comparing RNA extraction efficiency using a commercial kit or third-party silica columns. All extractions were performed in duplicate from the same 4-hour culture of *E. coli* using ThermoFisher GeneJET Kit protocol and buffers. In all cases, 5 min lysozyme treatment step (3 mg/mL in 100 μL TE buffer) was included. Samples were diluted 10X in TE buffer before absorbance measurement. Error bars are standard deviations. NOVEL and SHANG third-party columns performed better than the columns from GeneJET Kit. NOVEL columns produced the highest amount and best quality product with 260/280 and 260/230 ratios of 1.8 and 2.1, respectively. All third-party columns were obtained as free samples from the following companies: SHANG – Shanghai Changheng Industry & Trade CO., Ltd, Shanghai, China; NOVEL – NovelGene Biotech Corporation, Taipei, Taiwan; EPOCH – Epoch Life Science, Sugar Land, TX.
Figure S14: Comparing RNA extraction efficiency using a commercial kit or in-house buffers. All extractions were performed in duplicate from the same overnight culture of *E. coli* using columns from the Biobasic Total RNA Kit. Samples were diluted 10X in TE buffer before absorbance measurement. Error bars are standard deviations. All in-house buffers yielded a higher amount of product compared to commercial kits. LogSpin buffers produced the highest amount and best quality product with 260/280 and 260/230 ratios of 2 and 2.2, respectively. All in-house buffers were made, and extractions were performed following published protocols: LogSpin – Ref. (6); RNASwift – Ref. (7); EPOCH – Ref. (8). In all cases, 5 min lysozyme treatment step (3 mg/mL in 100 μL TE buffer) was included.
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