

Table S1. Most represented proteins on *S. flexneri* 1b OAg-positive and OAg-negative GMMA. CV: coefficient of variation. * t-test on difference between w/w values.

| <i>S. flexneri</i> 1b GMMA | | | | | | | |
|----------------------------|-------|--|------------------|-----|------------------|------|--------------------------|
| Accession number | MW | Protein | OAg ⁻ | | OAg ⁺ | | Significance p-value* |
| | | | % (w/w) | CV | % (w/w) | CV | |
| WP_000865562.1 | 41402 | porin OmpC [Enterobacteriaceae] | 29.1 | 5% | 33.3 | 1% | 0.129 |
| WP_005047463.1 | 37283 | porin OmpA [Enterobacteriaceae] | 32.8 | 6% | 23.4 | 1% | 0.086 |
| WP_001295296.1 | 18603 | outer membrane protein OmpX [Bacteria] | 5.8 | 20% | 4.9 | 7% | 0.451 |
| WP_000648420.1 | 8323 | murein lipoprotein Lpp [Bacteria] | 5.0 | 5% | 3.5 | 41% | 0.382 |
| WP_000735285.1 | 53799 | outer membrane channel protein TolC [Enterobacteriaceae] | 4.2 | 4% | 6.0 | 5% | 0.030 |
| WP_000597196.1 | 15602 | outer membrane lipoprotein SlyB [Proteobacteria] | 2.4 | 20% | 2.3 | 25% | 0.969 |
| WP_000742443.1 | 49381 | serine endoprotease DegP [Enterobacteriaceae] | 2.4 | 4% | 3.2 | 5% | 0.045 |
| WP_001295307.1 | 45956 | Tol-Pal system protein TolB [Proteobacteria] | 1.9 | 16% | 1.6 | 2% | 0.355 |
| WP_000249126.1 | 72755 | penicillin-binding protein activator LpoA [Enterobacteriaceae] | 1.5 | 1% | 0.6 | 116% | 0.282 |
| WP_001297320.1 | 36842 | outer membrane protein assembly factor BamC [Proteobacteria] | 1.4 | 4% | 2.3 | 4% | 0.013 |
| WP_001240896.1 | 90553 | outer membrane protein assembly factor BamA [Proteobacteria] | 1.3 | 2% | 1.8 | 3% | 0.027 |
| WP_000977934.1 | 39363 | porin OmpF [Enterobacteriaceae] | 1.2 | 5% | 2.1 | 2% | 0.007 |
| WP_011069568.1 | 80957 | ferric aerobactin receptor IutA [Enterobacteriaceae] | 0.9 | 17% | 1.0 | 9% | 0.382 |
| WP_001177043.1 | 41827 | outer membrane protein assembly factor BamB [Enterobacteriaceae] | 0.9 | 2% | 1.5 | 5% | 0.045 |
| WP_000163771.1 | 27831 | scaffolding protein MipA [Proteobacteria] | 0.5 | 43% | 0.6 | 16% | 0.714 |
| Tot (w/w) | | | 91.4 | | 88.1 | | |

Table S2. Most represented proteins on *S. flexneri* 2a OAg-positive and OAg-negative GMMA. CV: coefficient of variation, n.d.: not detected. * t-test on difference between w/w values.

| <i>S. flexneri</i> 2a GMMA | | | | | | | |
|----------------------------|-------|--|------------------|------|------------------|------|-----------------------|
| Accession number | MW | Protein | OAg ⁻ | | OAg ⁺ | | Significance p-value* |
| | | | % (w/w) | CV | % (w/w) | CV | |
| WP_000865562.1 | 41402 | porin OmpC [Enterobacteriaceae] | 30.5 | 14% | 31.2 | 13% | 0.887 |
| WP_005047463.1 | 37283 | porin OmpA [Enterobacteriaceae] | 31.7 | 2% | 16.1 | 2% | 0.006 |
| WP_000648420.1 | 8323 | murein lipoprotein Lpp [Bacteria] | 4.4 | 115% | 7.4 | 8% | 0.551 |
| WP_000742443.1 | 49381 | serine endoprotease DegP [Enterobacteriaceae] | 1.3 | 3% | 6.9 | 6% | 0.032 |
| WP_001295296.1 | 18603 | outer membrane protein OmpX [Bacteria] | 7.0 | 2% | 3.9 | 6% | 0.010 |
| WP_001240896.1 | 90553 | outer membrane protein assembly factor BamA [Proteobacteria] | 0.8 | 116% | 2.9 | 2% | 0.195 |
| WP_000597196.1 | 15602 | outer membrane lipoprotein SlyB [Proteobacteria] | 1.8 | 6% | 2.6 | 29% | 0.366 |
| WP_000838272.1 | 28928 | FKBP-type peptidyl-prolyl cis-trans isomerase [Enterobacteriaceae] | 1.4 | 2% | 2.1 | 6% | 0.064 |
| WP_000735285.1 | 53799 | outer membrane channel protein TolC [Enterobacteriaceae] | 0.9 | 5% | 1.9 | 1% | 0.014 |
| WP_001240896.1 | 39363 | porin OmpF [Enterobacteriaceae] | 0.8 | 116% | 2.9 | 2% | 0.195 |
| WP_001295307.1 | 45956 | Tol-Pal system protein TolB [Proteobacteria] | 0.7 | 114% | 1.6 | 1% | 0.387 |
| WP_000249126.1 | 72755 | penicillin-binding protein activator LpoA [Enterobacteriaceae] | 1.2 | 0.2% | 1.6 | 0.2% | < 0.001 |
| WP_000800457.1 | 47284 | peptidylprolyl isomerase SurA [Proteobacteria] | 0.7 | 2% | 1.3 | 4% | 0.037 |
| WP_001297320.1 | 36842 | outer membrane protein assembly factor BamC [Proteobacteria] | 0.6 | 1% | 1.1 | 6% | 0.064 |
| WP_001177043.1 | 41827 | outer membrane protein assembly factor BamB [Enterobacteriaceae] | 0.5 | 2% | 1.1 | 3% | 0.013 |
| WP_005049680.1 | 61019 | oligopeptide ABC transporter substrate-binding protein OppA [Enterobacteriaceae] | 0.6 | 4% | 1.0 | 2% | 0.003 |
| Tot (w/w) | | | 84.9 | | 85.6 | | |

Table S3. Most represented proteins on *S. flexneri* 3a OAg-positive and OAg-negative GMMA. CV: coefficient of variation, nd: not detected. * t-test on difference among w/w values.

| <i>S. flexneri</i> 3a GMMA | | | | | | | |
|----------------------------|-------|---|------------------|------|------------------|------|-----------------------|
| Accession number | MW | Description | OAg ⁻ | | OAg ⁺ | | Significance p-value* |
| | | | % (w/w) | CV | % (w/w) | CV | |
| WP_000865562.1 | 41402 | porin OmpC [Enterobacteriaceae] | 33.0 | 3% | 33.1 | 1% | 0.970 |
| WP_005047463.1 | 37283 | porin OmpA [Enterobacteriaceae] | 25.9 | 14% | 22.8 | 2% | 0.425 |
| WP_000742443.1 | 49381 | serine endoprotease DegP [Enterobacteriaceae] | 2.2 | 18% | 2.4 | 4% | 0.689 |
| WP_000648420.1 | 8323 | murein lipoprotein Lpp [Bacteria] | 7.0 | 29% | 7.0 | 2% | 0.988 |
| WP_001295296.1 | 18603 | outer membrane protein OmpX [Bacteria] | 5.3 | 32% | 3.4 | 0.4% | 0.351 |
| WP_001240896.1 | 90553 | outer membrane protein assembly factor BamA [Proteobacteria] | 2.6 | 24% | 1.8 | 1% | 0.338 |
| WP_000597196.1 | 15602 | outer membrane lipoprotein SlyB [Proteobacteria] | 2.3 | 55% | 2.9 | 1% | 0.606 |
| WP_000838272.1 | 28928 | FKBP-type peptidyl-prolyl cis- trans isomerase [Enterobacteriaceae] | 1.0 | 47% | 1.2 | 5% | 0.795 |
| WP_001295307.1 | 45956 | Tol-Pal system protein TolB [Proteobacteria] | 1.1 | 42% | 1.2 | 1% | 0.696 |
| WP_001177043.1 | 36842 | outer membrane protein assembly factor BamC [Proteobacteria] | 1.0 | 9% | 0.8 | 12% | 0.223 |
| WP_001297320.1 | 41827 | outer membrane protein assembly factor BamB [Enterobacteriaceae] | 1.0 | 22% | 0.9 | 0.3% | 0.667 |
| WP_000735285.1 | 53799 | outer membrane channel protein TolC [Enterobacteriaceae] | 2.7 | 3% | 2.3 | 2% | 0.034 |
| WP_000800457.1 | 47284 | peptidylprolyl isomerase SurA [Proteobacteria] | 0.7 | 128% | 0.6 | 6% | 0.889 |
| WP_001295306.1 | 18824 | peptidoglycan-associated lipoprotein Pal [Proteobacteria] | 0.6 | 120% | 1.3 | 4% | 0.382 |
| WP_000768975.1 | 40893 | glycerophosphodiester phosphodiesterase [Enterobacteriaceae] | 0.7 | 98% | 1.1 | 6% | 0.497 |
| WP_000737226.1 | 22928 | outer membrane protein OmpW [Enterobacteriaceae] | 1.3 | 65% | 1.8 | 4% | 0.559 |
| Tot (w/w) | | | 88.4 | | 84.5 | | |

Table S4. Most represented proteins on *S. flexneri* OAg-positive GMMA. CV: coefficient of variation, nd: not detected. *One way Anova or t test.

| OAg ⁺ GMMA | | | | | | | | | Significance p-value* |
|-----------------------|-------|--|-----------------------|------|-----------------------|------|-----------------------|------|--------------------------|
| Accession number | MW | Description | <i>S. flexneri</i> 1b | | <i>S. flexneri</i> 2a | | <i>S. flexneri</i> 3a | | |
| | | | % (w/w) | CV | % (w/w) | CV | % (w/w) | CV | |
| WP_000865562.1 | 41402 | porin OmpC [Enterobacteriaceae] | 33.3 | 1% | 31.2 | 13% | 33.1 | 1% | 0.657 |
| WP_005047463.1 | 37283 | porin OmpA [Enterobacteriaceae] | 23.4 | 1% | 16.1 | 2% | 22.8 | 2% | < 0.001 |
| WP_001295296.1 | 18603 | outer membrane protein OmpX [Bacteria] | 4.9 | 7% | 3.9 | 6% | 3.4 | 0.4% | 0.019 |
| WP_000648420.1 | 8323 | murein lipoprotein Lpp [Bacteria] | 3.5 | 41% | 7.4 | 8% | 7.0 | 2% | 0.041 |
| WP_000735285.1 | 53799 | outer membrane channel protein TolC [Enterobacteriaceae] | 6.0 | 5% | 1.9 | 1% | 2.3 | 2% | < 0.001 |
| WP_000597196.1 | 15602 | outer membrane lipoprotein SlyB [Proteobacteria] | 2.3 | 25% | 2.6 | 29% | 2.9 | 1% | 0.621 |
| WP_000742443.1 | 49381 | serine endoprotease DegP [Enterobacteriaceae] | 3.2 | 5% | 6.9 | 6% | 2.4 | 4% | 0.001 |
| WP_001295307.1 | 45956 | Tol-Pal system protein TolB [Proteobacteria] | 1.6 | 2% | 1.6 | 1% | 1.2 | 1% | < 0.001 |
| WP_000249126.1 | 72755 | penicillin-binding protein activator LpoA [Enterobacteriaceae] | 0.6 | 116% | 1.6 | 0.2% | nd | - | 0.270 |
| WP_001297320.1 | 36842 | outer membrane protein assembly factor BamC [Proteobacteria] | 2.3 | 4% | 1.1 | 6% | 0.9 | 0.3% | < 0.001 |
| WP_001240896.1 | 90553 | outer membrane protein assembly factor BamA [Proteobacteria] | 1.8 | 3% | 2.9 | 2% | 1.8 | 1% | < 0.001 |
| WP_000977934.1 | 39363 | porin OmpF [Enterobacteriaceae] | 2.1 | 2% | nd | - | nd | - | - |
| WP_011069568.1 | 80957 | ferric aerobactin receptor IutA [Enterobacteriaceae] | 1.0 | 9% | nd | - | nd | - | - |
| WP_001177043.1 | 41827 | outer membrane protein assembly factor BamB [Enterobacteriaceae] | 1.5 | 5% | 1.1 | 3% | 0.8 | 12% | 0.006 |
| WP_000163771.1 | 27831 | scaffolding protein MipA [Proteobacteria] | 0.6 | 16% | nd | - | nd | - | - |
| WP_000838272.1 | 28928 | FKBP-type peptidyl-prolyl cis- trans isomerase [Enterobacteriaceae] | nd | - | 2.1 | 6% | 1.2 | 5% | 0.029 |
| WP_000800457.1 | 47284 | peptidylprolyl isomerase SurA [Proteobacteria] | nd | - | 1.3 | 4% | 0.6 | 6% | 0.009 |
| WP_001295306.1 | 18824 | peptidoglycan-associated lipoprotein Pal [Proteobacteria] | nd | - | nd | - | 1.3 | 4% | - |
| WP_000768975.1 | 40893 | glycerophosphodiester phosphodiesterase [Enterobacteriaceae] | nd | - | nd | - | 1.1 | 6% | - |
| WP_000737226.1 | 22928 | outer membrane protein OmpW [Enterobacteriaceae] | nd | - | nd | - | 1.8 | 4% | - |
| WP_005049680.1 | 61019 | oligopeptide ABC transporter substrate-binding protein OppA [Enterobacteriaceae] | nd | - | 1.0 | 2% | nd | - | - |
| Total (w/w) | | | 88.1 | | 82.6 | | 84.5 | | |

Table S5. Most represented proteins on *S. flexneri* OAg-negative GMMA. CV: coefficient of variation, nd: not detected. *One way Anova or t test.

| OAg GMMA | | | | | | | | | Significance p-value* |
|---------------------|-------|--|-----------------------|-----|-----------------------|------|-----------------------|------|--------------------------|
| Accession number | MW | Description | <i>S. flexneri</i> 1b | | <i>S. flexneri</i> 2a | | <i>S. flexneri</i> 3a | | |
| | | | % (w/w) | CV | % (w/w) | CV | % (w/w) | CV | |
| WP_000865562.1 | 41402 | porin OmpC [Enterobacteriaceae] | 29.1 | 5% | 30.5 | 14% | 33.0 | 3% | 0.448 |
| WP_005047463.1 | 37283 | porin OmpA [Enterobacteriaceae] | 32.8 | 6% | 31.7 | 2% | 25.9 | 14% | 0.115 |
| WP_001295296.1 | 18603 | outer membrane protein OmpX [Bacteria] | 5.8 | 20% | 7.0 | 2% | 5.3 | 32% | 0.466 |
| WP_000648420.1 | 8323 | murein lipoprotein Lpp [Bacteria] | 5.0 | 5% | 4.4 | 115% | 7.0 | 29% | 0.718 |
| WP_000735285.1 | 53799 | outer membrane channel protein TolC [Enterobacteriaceae] | 4.2 | 4% | 0.9 | 5% | 2.7 | 3% | < 0.001 |
| WP_000597196.1 | 15602 | outer membrane lipoprotein SlyB [Proteobacteria] | 2.4 | 20% | 1.8 | 6% | 2.3 | 55% | 0.727 |
| WP_000742443.1 | 49381 | serine endoprotease DegP [Enterobacteriaceae] | 2.4 | 4% | 1.3 | 3% | 2.2 | 18% | 0.040 |
| WP_001295307.1 | 45956 | Tol-Pal system protein TolB [Proteobacteria] | 1.9 | 16% | 0.7 | 114% | 1.1 | 42% | 0.249 |
| WP_000249126.1 | 72755 | penicillin-binding protein activator LpoA [Enterobacteriaceae] | 1.5 | 1% | 1.2 | 0% | nd | - | 0.032 |
| WP_001297320.1 | 36842 | outer membrane protein assembly factor BamC [Proteobacteria] | 1.4 | 4% | 0.6 | 1% | 1.0 | 22% | 0.020 |
| WP_001240896.1 | 90553 | outer membrane protein assembly factor BamA [Proteobacteria] | 1.3 | 2% | 0.8 | 116% | 2.6 | 24% | 0.151 |
| WP_000977934.1 | 39363 | porin OmpF [Enterobacteriaceae] | 1.2 | 5% | nd | - | nd | - | - |
| WP_011069568.1 | 80957 | ferric aerobactin receptor IutA [Enterobacteriaceae] | 0.9 | 17% | nd | - | nd | - | - |
| WP_001177043.1 | 41827 | outer membrane protein assembly factor BamB [Enterobacteriaceae] | 0.9 | 2% | 0.5 | 2% | 1.0 | 9% | 0.005 |
| WP_000163771.1 | 27831 | scaffolding protein MipA [Proteobacteria] | 0.5 | 43% | nd | - | nd | - | - |
| WP_000838272.1 | 28928 | FKBP-type peptidyl-prolyl cis-trans isomerase [Enterobacteriaceae] | nd | - | 1.4 | 2% | 1.0 | 47% | 0.538 |
| WP_000800457.1 | 47284 | peptidylprolyl isomerase SurA [Proteobacteria] | nd | - | 0.7 | 2% | 0.7 | 128% | 0.986 |
| WP_005049680.1 | 61019 | oligopeptide ABC transporter substrate-binding protein OppA [Enterobacteriaceae] | nd | - | 0.6 | 4% | nd | - | - |
| WP_001295306.1 | 18824 | peptidoglycan-associated lipoprotein Pal [Proteobacteria] | nd | - | nd | - | 0.6 | 120% | - |
| WP_000768975.1 | 40893 | glycerophosphodiester phosphodiesterase [Enterobacteriaceae] | nd | - | nd | - | 0.7 | 98% | - |
| WP_000737226.1 | 22928 | outer membrane protein OmpW [Enterobacteriaceae] | nd | - | nd | - | 1.3 | 65% | - |
| Total (w/w) | | | 91.4 | | 84.1 | | 88.4 | | |

Table S6. Composition of Chemically Defined Medium.

| | | Concentration | | | |
|---------------------------------|-------------------|------------------|-------------------------|-------------------------|-------------------------|
| Reagent | Supplier Code# | <i>S. sonnei</i> | <i>S flexneri</i> 1b | <i>S flexneri</i> 2a | <i>S flexneri</i> 3a |
| Sulfuric Acid | VWR -1.00731.1000 | [13] | 20 µL/kg | 20 µL/kg | 20 µL/kg |
| Manganese chloride tetrahydrate | SIGMA -M8054 | | 15 mg/kg | 15 mg/kg | 15 mg/kg |
| Copper Chloride dihydrate | SIGMA -307483 | | 1.5 mg/kg | 1.5 mg/kg | 1.5 mg/kg |
| Boric Acid solution 4% | SIGMA-31144 | | 75 mg/kg | 75 mg/kg | 75 mg/kg |
| Sodium Molybdate Dihydrate | SIGMA-M1651 | | 2.5 mg/kg | 2.5 mg/kg | 2.5 mg/kg |
| Zinc acetate dihydrate | SIGMA -383058 | | 13 mg/kg | 13 mg/kg | 13 mg/kg |
| Hexammine cobalt(III) chloride | SIGMA-H7891 | | 2.5 mg/kg | 2.5 mg/kg | 2.5 mg/kg |
| Ferric Citrate | SIGMA -F3388 | | 24.5 mg/kg | 24.5 mg/kg | 24.5 mg/kg |
| Magnesium Sulfate heptahydrate | SIGMA-63138 | | 493.0 mg/kg | 493.0 mg/kg | 493.0 mg/kg |
| Thiamine Hydrochloride | SIGMA-T4562 | | 50 mg/kg | 50 mg/kg | 50 mg/kg |
| Nicotinic Acid | SIGMA -N0761 | | 10 mg/kg | 10 mg/kg | 10 mg/kg |
| Potassium Phosphate Monobasic | SIGMA-P5655 | | 13.3 g/kg | 13.3 g/kg | 13.3 g/kg |
| Di-Ammonium HydrogenPhosphate | SIGMA-215996 | | 4 g/kg | 4 g/kg | 4 g/kg |
| Citric Acid Monohydrate | SIGMA-C1909 | | 1.7 g/kg | 1.7 g/kg | 1.7 g/kg |
| Dextrose monohydrate | SIGMA -D9559 | | 5 g/kg | 5 g/kg | 5 g/kg |
| L-Aspartic acid | SIGMA -A7219 | | 2.5 g/kg | 2.5 g/kg | 2.5 g/kg |
| L-Methionine | SIGMA-M5308 | | 0.16 g/kg | 0 g/kg | 0.16 g/kg |

| | | | | | |
|---|-------------|--|-----------|--------|-----------|
| L-Asparagine | SIGMA-A4159 | | 0.06 g/kg | 0 g/kg | 0.06 g/kg |
| L-Threonine | SIGMA-T8441 | | 0.22 g/kg | 0 g/kg | 0.22 g/kg |
| L-Lysine monohydrate | SIGMA-L5510 | | 0.60 g/kg | 0 g/kg | 0.67 g/kg |
| L-Glutamic acid monosodium salt monohydrate | SIGMA-49621 | | 1.78 g/kg | 0 g/kg | 1.78 g/kg |

Table S7. HPLC-RP gradient. Solvent A: 5% Acetonitrile, 15% Isopropyl alcohol, 0.3% TFA in water (by volume); Solvent B: 80% Acetonitrile, 15% Isopropyl alcohol, 0.3% TFA in water (by volume); Solvent C: Isopropyl alcohol. Column temperature: 70° C.

| Time | Flow (mL/min) | %A | %B | %C | Curve |
|---------|------------------|-----|-----|-----|---------|
| Initial | 0.2 | 100 | 0 | 0 | Initial |
| 2 | 0.2 | 100 | 0 | 0 | 6 |
| 8 | 0.2 | 70 | 30 | 0 | 6 |
| 13 | 0.2 | 55 | 45 | 0 | 6 |
| 24 | 0.2 | 0 | 100 | 0 | 6 |
| 28 | 0.2 | 0 | 100 | 0 | 6 |
| 34 | 0.2 | 0 | 0 | 100 | 6 |
| 44 | 0.2 | 0 | 0 | 100 | 6 |
| 49 | 0.2 | 100 | 0 | 0 | 6 |
| 69 | 0.2 | 100 | 0 | 0 | 6 |

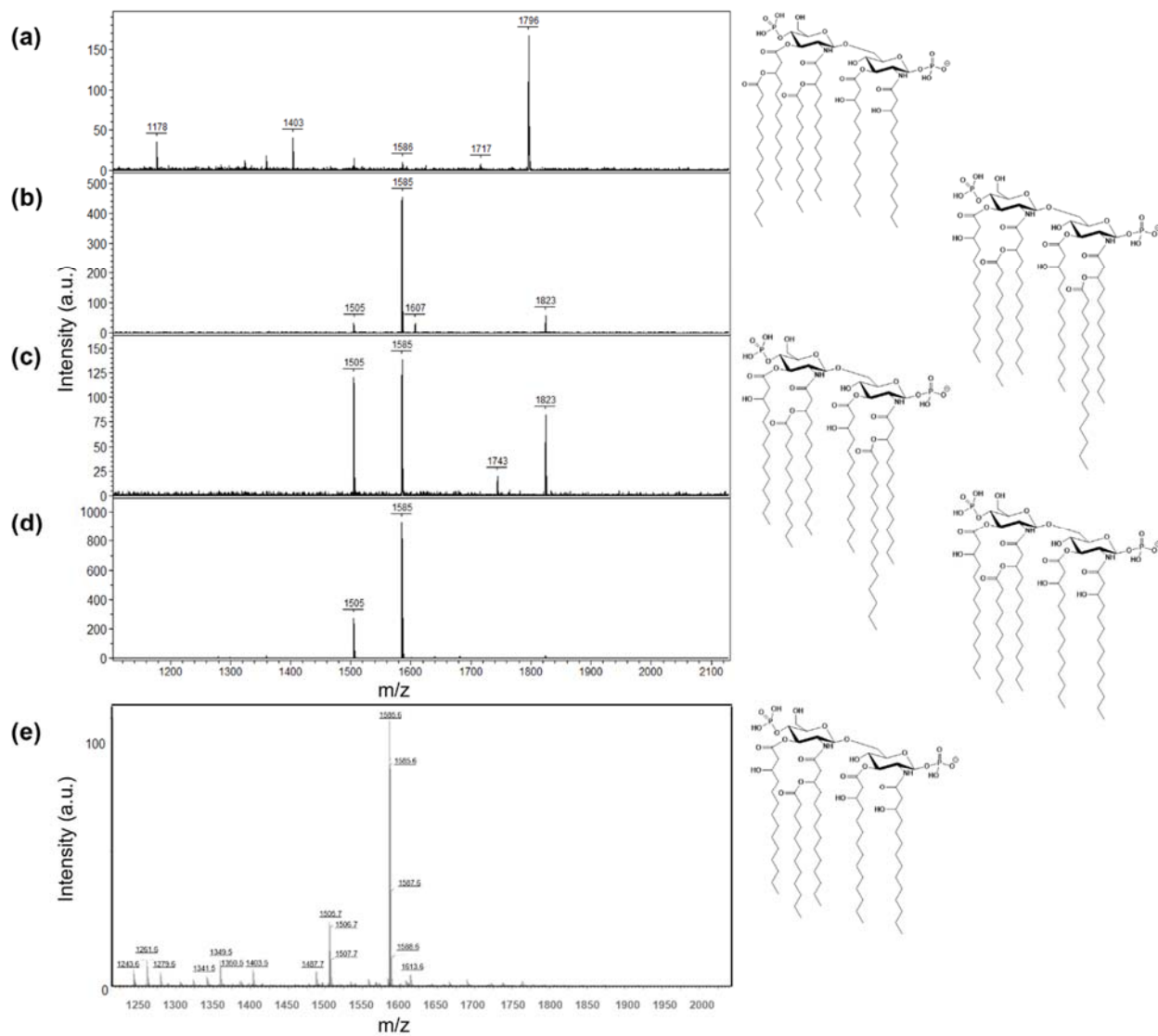


Figure S1. MALDI-TOF spectra and structures of lipid A. (a) wild type lipid A, (b) *S. flexneri* 1b GMMA lipid A, (c) *S. flexneri* 2a GMMA lipid A, (d) *S. flexneri* 3a GMMA lipid A, (e) *S. sonnei* GMMA lipid A.

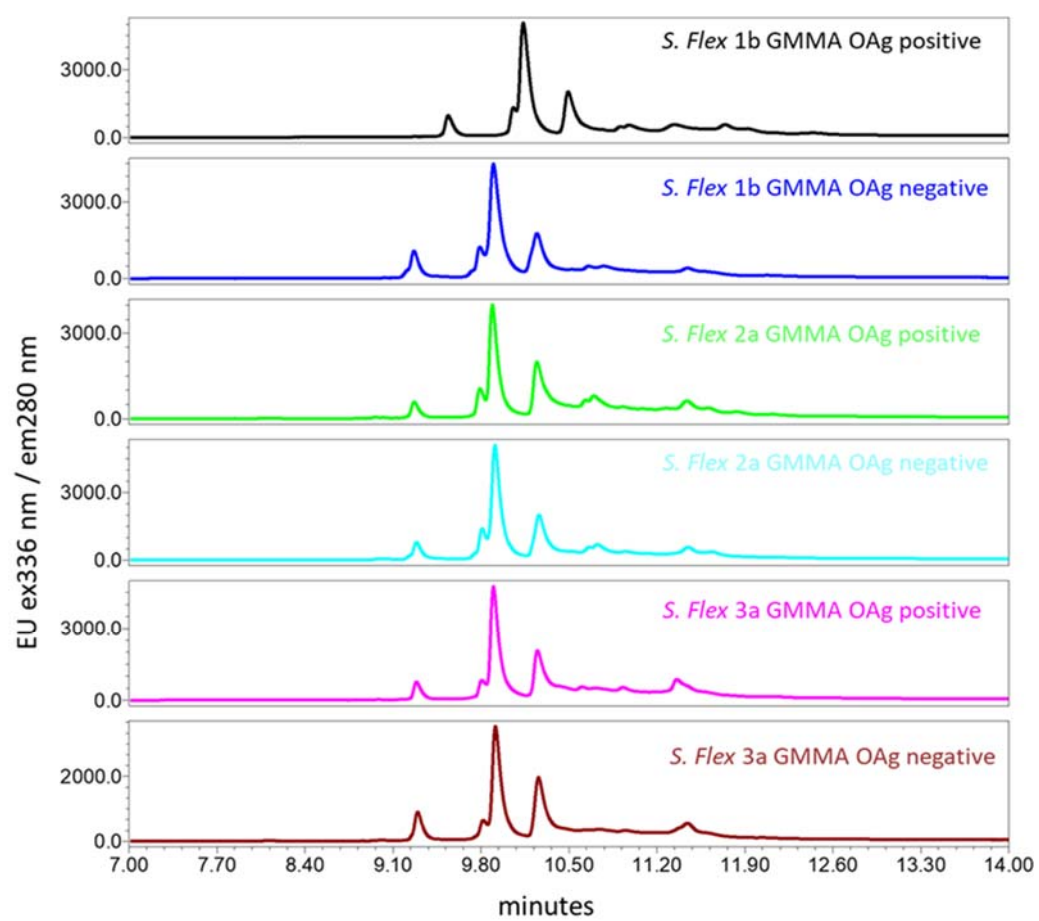


Figure S2. GMMA HPLC-RP protein profile.