QIAxcel images of the 14 sets of multiplex PCR assays targeting 145 serogroups of Shiga toxin-producing *Escherichia coli* for testing specificity of each assay set. Each assay was run with DNA from pooled cultures of the targeted serogroup (PC), DNA from pooled cultures for the other 13 sets and top-7 plus O104, and negative control (NC), which included all reagents except the DNA template.

mPCR assay set 1



mPCR assay set 2



O group	bp
O128	768
O138	696
O123	619
O120	535
O119	421
O13	364
O175	343
O171	281
O22	246
O5	176

Set 1	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +104	PC	NC

mPCR assay set 3



O group	bp
O6	783
O98	675
O181	595
O75	511
O33	413
O116	355
O150	313
O79	266
O25	230

Set 1	Set 2	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 4



O group	bp
O78	992
O2	819
O146	640
O76	533
O178	495
O126	465
O113	419
O118	344
O15	288
O147	230

Set 1	Set 2	Set 3	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mpCR assay set 5



O group	bp
O62/O68	906
O7	753
O163	596
O136	528
O8	448
O92	375
O87	306
O55	262
O20/O137	204

Set 1 **Set 2** **Set 3** **Set 4** **Set 6** **Set 7** **Set 8** **Set 9** **Set 10** **Set 11** **Set 12** **Set 13** **Set 14** **Top-7 +O104** **PC** **NC**

mPCR assay set 6



O group	bp
O141	880
O153	741
O132	652
O130	567
O108	515
O96	457
O88	394
O107/117	357
O74	303
O38	253
O39	201
O115	158

Set 1	Set 2	Set 3	Set 4	Set 5	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 7



Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

O group	bp
O70	863
O53	735
O69	649
O51	566
O17/44/73/77/106	500
O43	445
O40	396
O37	353
O35	305
O28 (O42/O20ac)	255
O18 (18ab/18ac)	199
O1	152

mPCR assay set 8



O group	bp
O139	859
O125 (125ab/125ac)	652
O124/O164	570
O90/O127	498
O102	453
O105	407
O85	353
O82	301
O81	248
O148	201
O140	155

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 9



O group	bp
O169	865
O161	646
O154	499
O114	396
O110	346
O93	299
O149	253
O49	197
O21	145

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 10	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 10



O group	bp
O165	735
O160	655
O182	566
O179	505
O46/O134	455
O177	395
O176	356
O174	317
O172	278
O170	233
O159	202
O152	150

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 11	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 11



O group	bp
O131	655
O19	574
O16	505
O63	455
O23	403
O29	348
O101/O162	309
O112ab	270
O11	225
O10	187
O3	145

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 12	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 12



O group	bp
O41	942
O48	793
O142	538
O143	500
O60	443
O27	382
O54	351
O9	309
O56	250

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 13	Set 14	Top-7 +O104	PC	NC

mPCR assay set 13



O group	bp
O58	1046
O12	885
O64	727
O166	462
O167	403
O83	362
O133	294

Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 14	Top-7 +O104	PC	NC

mPCR assay set 14

