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Performance of copan WASP for routine urine microbiology

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Abstract: This study compared manual workup of urine clinical samples with fully automated WASPLab processing. As a first step two different inocula (1 and 10 l) and different streaking patterns were compared using WASP and Inoqula BT™ instrumentation. A 10 l inoculum produced significantly more single colonies than a 1 l inoculum and automated streaking yielded significantly more single colonies as compared to manual streaking on whole plates ($p < 0.001$). In a second step, 379 clinical urine samples were evaluated using WASP and manual workup. Average numbers of detected morphologies, recovered species, and CFU/ml of all 379 urine samples showed excellent agreement of WASPLab and manual workup. The percentage of clinical categorization of urine samples as "positive" or "negative" did not differ between automated and manual work-flow but within the positive samples automated processing by WASPLab resulted in the detection of more potential pathogens. In summary, the present study demonstrates that i) the streaking pattern, i.e. primarily the number of zigzags/length of streaking lines, is critical for optimizing the number of single colonies yielded from primary cultures of urine samples, ii) automated streaking by the WASP instrument was superior to manual streaking regarding the number of single colonies yielded, (for 32.2%) iii) automated streaking leads to higher numbers of detected morphologies (for 47.5%), species (for 17.4%) and pathogens (for 3.4%). The results of this study point to an improved quality of microbiological analyses and laboratory reports when using automated sample processing by WASP and WASPLab.

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1 **Performance of Copan WASP for Routine Urine Microbiology**

2

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22 **Abstract**

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24 WASPLab processing. As a first step two different inocula (1 and 10 μ l) and different
25 streaking patterns were compared using WASP and Inoqula BT™ instrumentation. A 10
26 μ l inoculum produced significantly more single colonies than a 1 μ l inoculum and
27 automated streaking yielded significantly more single colonies as compared to manual
28 streaking on whole plates ($p < 0.001$). In a second step, 379 clinical urine samples were
29 evaluated using WASP and manual workup. Average numbers of detected
30 morphologies, recovered species, and CFU/ml of all 379 urine samples showed
31 excellent agreement of WASPLab and manual workup. The percentage of clinical
32 categorization of urine samples as “positive” or “negative” did not differ between
33 automated and manual work-flow but within the positive samples automated processing
34 by WASPLab resulted in the detection of more potential pathogens. In summary, the
35 present study demonstrates that i) the streaking pattern, i.e. primarily the number of
36 zigzags/length of streaking lines, is critical for optimizing the number of single colonies
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40 morphologies (for 47.5%), species (for 17.4%) and pathogens (for 3.4%). The results of
41 this study point to an improved quality of microbiological analyses and laboratory
42 reports when using automated sample processing by WASP and WASPLab.

43 **Introduction**

44 In recent years, clinical microbiology has been faced with dramatic changes, as
45 full laboratory automation (FLA) has started to enter diagnostic laboratories. This trend
46 to automation will affect to various extents economic efficiency, standardization, and
47 time-to-result of laboratory procedures (16). To date, clinical microbiology is still
48 predominately based on manual sample processing. Compared to clinical chemistry,
49 microbiological specimens display a significantly higher degree of complexity (2). Thus,
50 for many years the general perception was that clinical microbiology would be far too
51 complex to allow for automated processing, and that robots would not be able to replace
52 human operators. However, it has been demonstrated that automated inoculation of
53 samples can be superior to manual processing and that automated reading of disk
54 diffusion agar plates significantly increases precision of results (4, 5, 9, 13). Thus,
55 automated sample processing promises an improved standardization of sample
56 processing, incubation times, and plate reading protocols.

57 FLA systems have been developed by several companies. Currently, there are
58 two FLA solutions available, i.e. BD Kiestra total laboratory automation (TLATM; BD
59 Kiestra B.V., Drachten, Netherlands) and WASPLab (Copan Italia S.p.A., Brescia, Italy).
60 These systems use robotic systems for handling specimen containers and primary
61 culture inoculation specifically the Kiestra Inoqua and the Copan WASP. The
62 inoculated media are moved to automated incubators by conveyor belts with integrated
63 camera systems to capture plate images at given time points which is henceforth
64 referred to in this article as WASPLab. Currently, digital plate reading (DPR) is still
65 depending on highly skilled technologists, who can read plates “virtually” without

66 physical interaction (15). First studies on FLA solutions suggest that productivity
67 indicators can be improved and diagnostic processes can be accelerated (3, 6, 7, 14).

68 In the present study, we compared workflows for urine sample processing using
69 the fully automated WASP and WASPLab systems and manual standard procedures.
70 Inoculation procedures critically influence further procedures such as specimen
71 identification (ID) and antimicrobial susceptibility testing (AST), mainly by the ability to
72 generate single colonies suitable for further processing. Therefore, both streaking
73 pattern and inoculation volume should be well evaluated prior to compare total manual
74 and automated workflows. Different guidelines exist for urine specimen inoculation, e.g.
75 U.S. and Canadian laboratories routinely plate 1 μl of urine specimens, whereas
76 European guidelines suggest 10 μl (5, 18). Therefore, this study was subdivided in two
77 parts: In part one, the ability to generate single colonies was compared for i) manual
78 and automated workflows and ii) for different automated streaking patterns using pure
79 and mixed cultures and different inocula (1 μl and 10 μl) in order to determine the
80 optimal streaking pattern. Part two of the present study compared total manual and
81 automated workflows regarding i) the number of detected CFU, ii) the number of
82 detected morphologies, iii) the number of recovered species, and iv) the number of
83 follow-up testing after initial plate reading, i.e. the number of identifications and
84 susceptibility tests, in 379 clinical urine samples.

85

86 **Materials and Methods**

87 **Bacterial strains, clinical samples and growth conditions.** Urine samples and
88 bacterial strains isolated from patient specimens used in this study were collected from

89 January until April 2015 in the clinical laboratory of the Institute of Medical Microbiology,
90 University of Zurich. If not stated otherwise bacterial cultures were incubated at 35 °C
91 ± 2 °C and 7.5% CO₂ for 16h-20h.

92 **Quantitative analysis of streaking pattern/single colony count.** Manual, semi and
93 fully automated quantitative streaking patterns were evaluated by counting the number
94 of single colonies produced on whole plates. Manual streaking was always performed
95 by the same investigator with a 1 μ l and a 10 μ l calibrated plastic inoculation loop
96 (Copan Italia S.p.A., Brescia, Italy) resulting in 9-10 zigzag lines (Figure 3A). For the
97 semi-automated streaking, a “ball-based” benchtop Inoqula BT™ (BD Kiestra,
98 Drachten, Netherlands) was used with a 1 μ l and 10 μ l inoculum and the zigzag liquid
99 streaking pattern resulting in 20 zigzag lines (Figure 3A). In this study, 1 μ l and 10 μ l
100 inocula were manual pipetted for Inoqula BT. Furthermore, 1 μ l was taken in
101 consideration as per term of comparison but it has to be noted that the BD Kiestra
102 solutions cannot handle 1 μ l. The fully automated streaking was performed by the “loop-
103 based” WASP (Copan Italia S.p.A.) with two different streaking patterns; the single
104 streak type 2 (SST2, 11 zigzag lines) and the single streak type 6 (SST6, 27 zigzag
105 lines, Figure 3A). Bacterial suspensions of McFarland 0.5, corresponding to $\sim 1.5 \times 10^8$
106 colony forming units per ml (CFU/ml), were serial diluted in sodium borate/formate
107 containing BD Vacutainer (BD, Franklin Lakes, NJ, USA) and plated onto Columbia 5 %
108 sheep blood agar (COS, bioMérieux SA, Marcy l’Etoile, France) for monomicrobial
109 cultures or chromogenic UriSelect™ 4 agar (URI4, Bio-Rad Laboratories, Hercules, CA,
110 USA) for polymicrobial cultures. Pure *Escherichia coli* and enterococci cultures were
111 diluted to 10^4 - 10^6 CFU/ml and mixed cultures were prepared with *E. coli* ranging from

112 10^4 to 10^6 CFU/ml together with 10^6 enterococci and 10^6 coagulase negative
113 staphylococci (CoNS). The single colony counting was done manually by eye by the
114 same investigator.

115 **Head to head comparison of urine samples.** In total, 379 clinical urine samples were
116 processed in parallel by the fully automated WASPLab (Copan Italia S.p.A.) workflow
117 (inoculation, incubation, image acquisition, image analysis) and the manual routine
118 procedures of our clinical laboratory. 1 μ l sample inoculum used in the manual routine
119 workflow was compared to 1 μ l (121 samples) and 10 μ l loop (379 samples) of the
120 WASP. Urine cultures were plated onto COS, URI4 and Columbia colistin-nalidixic acid
121 (CNA) agar 5 % sheep blood (bioMérieux SA).

122 Following manual inoculation agar plates were analyzed after one and two days of
123 incubation and morphologies, CFU count, and recovered species were recorded.
124 Material was reported negative when there was no growth or growth of a non-relevant
125 pathogen after two days of incubation. Materials were reported positive, if there was a
126 relevant pathogen in a relevant quantity, and if treatment was indicated. The sample
127 evaluation algorithm used resembles the diagnostic approach as suggested by the
128 Cumitech guidelines (12). Classification of the relevant pathogens was done as reported
129 elsewhere (10).

130 For WASP inoculation, 1 μ l and 10 μ l volumes of the urine sample were streaked with
131 the SST6 streaking pattern. Images were taken using WASPLab imaging after 16 and
132 38 h incubation at $35 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ and 7.5% CO_2 and analyzed by the same laboratory
133 personnel. After 16 h photos were read, morphologies (i.e. visually different colony
134 types), and CFU counts were noted. Plates were re-read after 38 h incubation. Colonies

135 with additional morphology were identified. Samples were categorized as negative and
136 positive according to the same criteria as for the manual reading procedure. Besides the
137 number of different morphologies, CFU counts and recovered species (i.e. taxonomic
138 entities as reported by MALDI-TOF-MS identification), the number of follow-up tests,
139 such as susceptibility tests and matrix-assisted laser desorption ionization-time of flight
140 mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany)
141 identifications, were recorded for comparison.

142 Plate reading of manually examined specimens was performed by different laboratory
143 personnel by visual inspection directly; plates of WASP-prepared samples were read by
144 a single technician with the WASPLab, who also did the head-to-head comparison of
145 the final dataset.

146 **Technical settings of WASPLab.** Automated images of COS and CNA plates were
147 recorded using three different light settings (Top-light, Dark-field, and a combination of
148 Top and Back-light). URI4 images were recorded with Top-light settings only. The
149 WASPLab installation used WASPCore version 3.1.0. The settings were conserved
150 throughout the whole study duration.

151 **Statistical analysis.** Statistical analysis was performed using the IBM SPSS statistic
152 software, version 20 (SPSS Inc., Chicago, IL, USA). The normality of the data was
153 assessed with the Kolmogorov-Smirnov and Shapiro-Wilk test, followed by the paired t-
154 test and the non-parametric Wilcoxon signed rank test for the comparison of loop size
155 and streaking pattern for pure cultures. A cutoff value of 0.05 was considered as
156 statistically significant. For the mixed cultures, overall differences were tested using the
157 Kruskal-Wallis and the one way ANOVA with Bonferroni post hoc test to control for

158 alpha inflation. Non-parametric Follow-up tests were conducted using the Mann-
159 Whitney U test for pairwise comparison with Bonferroni correction applied for multiple
160 comparisons with an alpha level of 0.05 divided by possible number of comparisons i.e.
161 0.0083 ($=0.05/6$) for streaking pattern and 0.0167 ($=0.05/3$) for concentration as
162 independent variables. To compare the proportion between manual and WASP
163 processing of the head to head comparison the Wilcoxon signed rank test and a
164 crosstab chi-square test of independence were performed. Proportions do differ
165 significantly from each other at the level of 0.05.

166 **Results**

167 As a first step two different inocula (1 μ l and 10 μ l) and different streaking
168 patterns (one manual, two fully automated WASP/"loop-based", and one semi-
169 automated Inoqua/"ball-based") were evaluated with pure cultures of *E. coli* and
170 enterococci using CFU counts of 10^4 /ml, 10^5 /ml, and 10^6 /ml, with the view to identify the
171 loop-streaking pattern combination which yields the highest number of single colonies
172 isolated (Figure 2, Figures S1 and S2).

173 We also studied, mixed inocula containing 10^6 CFU/ml enterococci, 10^6 CFU/ml
174 CoNS, and *E. coli* with CFU/ml ranging from 10^4 CFU/ml to 10^6 CFU/ml with the view to
175 mimic mixed clinical samples of a leading pathogen and residual flora.

176 ***Evaluation of the optimal inoculation volume***

177 The 10 μ l inoculum produced significantly more single colonies than the 1 μ l
178 inoculum on whole plates for both *E. coli* and enterococci when using CFU counts
179 between 10^4 /ml and 10^5 /ml. This finding was independent from the streaking pattern
180 ($p < 0.001$, Figure 2, B and C). In contrast, no significantly higher recovery of single
181 colonies was observed for *E. coli* CFUs of 10^6 /ml when using a 10 μ l versus 1 μ l
182 inoculum and when comparing manual streaking and the Inoqua ($p = 0.820$, and
183 $p = 0.713$, respectively) (Figure 2, B and C). However, WASP inoculation retained a
184 significantly higher recovery of single colonies when applying the 10 μ l inoculum
185 ($p < 0.001$, Figure 2, B and C) and manual streaking.

186 Analysis of mixed cultures of *E. coli*, enterococci, and CoNS basically resembled
187 the results of pure cultures (Figure 3): The 10 μ l inoculum resulted in the recovery of

188 significantly more single colonies than the 1 μ l inoculum for manual streaking, WASP
189 SST6 and the Inoqula ($p < 0.001$).

190 ***Evaluation of the optimal streaking pattern***

191 For pure cultures of *E. coli* and enterococci the WASP streaking pattern SST2
192 (11 zigzag) produced less single colonies than manual streaking for 6 of the 12
193 inoculum-CFU combinations, thereof 5 significantly ($p < 0.001$, Figure 2, B and C). In
194 contrast, WASP streaking pattern SST6 (27 zigzag) and Inoqula (20 zigzag) produced
195 significantly more single colonies than manual streaking for the majority of inoculum-
196 CFU combinations ($p < 0.001$, Figure 2, B and C). For 8 out of 12 inoculum/CFU
197 combinations the WASP SST6 streaking pattern produced significantly more single
198 colonies as compared to Inoqula, WASP SST2 or manual streaking ($p < 0.001$, Figure 2,
199 B and C).

200 For mixed cultures of *E. coli*, enterococci, and CoNS both WASP SST6 and
201 Inoqula yielded significantly more single colonies as compared to manual streaking
202 ($p < 0.001$). No significant difference in the recovery of single colonies was found for
203 mixed inocula when comparing WASP SST6 and Inoqula ($p = 0.071$) for the 10 μ l
204 inoculum, whereas for the 1 μ l inoculum Inoqula BT produced significantly more
205 colonies ($p = 0.001$).

206 ***Head to head comparison of clinical samples***

207 To study the performance of the WASP and WASPLab in the routine
208 microbiological work up we evaluated fully automated WASP processing (streaking,
209 incubation and reading) with manual processing for 379 clinical urine samples. Based
210 on the evaluation of optimal inoculation and streaking pattern (see above), a 10 μ l
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211 inoculum combined with SST6 (27 zigzag) streaking pattern was chosen for this
212 evaluation. We found an agreement between automated WASP processing and manual
213 workup for detected morphologies, recovered species, and CFU/ml, in 177 (46.7%), 287
214 (75.7%), and 239 (63.1%), of all 379 samples. However, WASP yielded significantly
215 higher numbers of morphologies and species recovered as compared to manual
216 processing in 180 (47.5%), and 66 (17.4%) of the 379 samples ($p < 0.001$, Table 1,
217 Figure 4, A and B). For 122 (32.2%) of the 379 samples, WASP workup (10 μ l
218 inoculum) indicated a higher CFU count/ml than manual handling (mean increase of
219 1 \log_{10}).

220 To exclude a bias due to the higher inoculum used in WASP processing, as
221 compared to manual processing, a subset of 121 samples were WASP-inoculated using
222 a 1 μ l loop. No significant difference in CFU count/ml was detected between automated
223 and manual workup, when inoculum was equally set to 1 μ l ($p = 0.176$, Table 2, Figure 4
224 C). However, the percentage of samples which displayed higher numbers of
225 morphologies and higher numbers of recovered species was still significantly higher
226 when using automated sample processing, as compared to manual workup (42/121, i.e.
227 34.7%, and 18/121, i.e. 14.9% of samples, respectively, $p < 0.001$, Table 2, Figure 4 A
228 and B).

229 Automated processing lead to the detection of more potential pathogens with
230 both the 10 μ l and the 1 μ l inoculum (3.4% and 9.1% of all 379 and 121 urine samples
231 processed with the corresponding inoculum, respectively, Table 2).

232 The percentage of urine samples categorized in clinical reports as “positive”, i.e.
233 treatment indicated, or “negative”, i.e. no treatment indicated, did differ but not

234 statistically significantly between manual or automatic handling. Overall, 37.2% and
235 40.4% of 379 samples were categorized as “positive” for manual and automated workup
236 respectively, while 62.8 and 59.6% were categorized as “negative” (Table 1). This ratio
237 was not affected by using different loop sizes for inoculation (compare to Table 2).

238 The significantly higher number of morphologies detected by WASPLab
239 processing lead to significantly more MALDI-TOF based identifications. However, the
240 number of subsequent antibiotic susceptibility tests performed was found not
241 significantly different between automated and manual workup, irrespective of whether
242 inoculation was done using a 10 μ l or 1 μ l loop (Tables 1 and 2).

243 **Discussion**

244 ***Evaluation of the optimal inoculum***

245 Traditionally, North American clinical laboratories use a 1 μ l inoculum for urine
246 specimens, while European laboratories inoculate 10 μ l as the primary inoculum
247 according to various guidelines (8, 11, 12, 17, 18). General arguments between the two
248 inoculation volumes are a potentially decreased sensitivity using a 1 μ l inoculum and the
249 missing of pathogens due to a potential lack of single colonies using a 10 μ l inoculum
250 for high CFU counts. We aimed at determining the optimal inoculum for the majority of
251 CFU/pathogen combinations that are most frequently encountered in the clinical
252 laboratory. *E. coli* was chosen as the most prevalent uropathogen (1). Enterococci and
253 CoNS were selected to represent the most frequently encountered Gram-positive
254 species in urine specimens (10). On whole plates the 10 μ l inoculum was demonstrated
255 to be superior to the 1 μ l inoculum in order to maximize the frequency of single colony
256 recovery, particularly for CFUs of $\leq 10^6$ /ml. As positivity rates and the number of
257 potential pathogens did not differ between the 1 μ l and the 10 μ l inoculum, the
258 abovementioned concerns on decreased sensitivity (1 μ l inoculum) and missing of
259 pathogens (10 μ l inoculum) could not be confirmed. A higher frequency of single colony
260 recovery using automated inoculation may have contributed to the increased detection
261 of potential pathogens (see also "Head to head comparison of clinical samples").
262 However, the effect of single colony recovery and improved visual inspection by high
263 resolution imaging on sample categorization cannot be distinguished in this study.

264 ***Evaluation of the optimal streaking pattern***

265 For pure cultures, WASP SST6 streaking was superior to both WASP SST2 and
266 InoqulaA streaking with respect to maximize the recovery of single colonies,
267 independently of the inoculum volume. For mixed cultures, single colony counts
268 generated by WASP SST6 and InoqulaA streaking did not differ significantly using a 10 μ l
269 inoculum ($p=0.071$). However, the number of samples investigated was limited, and a
270 larger follow up study may reveal statistically significant results given the borderline p -
271 value. The bench-top InoqulaA in combination with manual pipetting produced more
272 colonies as compared to the SST6 for the 1 μ l inoculum ($p=0.001$). However, the fully
273 automated InoqulaA cannot handle inoculation volumes lower than 10 μ l. In part, these
274 findings are in contrast to a recent study that reported the InoqulaA to be superior to
275 WASP streaking with respect to the recovery of single colonies (4, 5, 13). In our study,
276 this effect was shown mainly for exceptionally high CFU counts of 10^7 to 10^8 CFU/ml.
277 However, as the vast majority of clinical specimens ($228/306 = 74.5\%$ in this study)
278 contain up to 10^5 CFU/ml. Here, for pure cultures WASP streaking was even superior to
279 the InoqulaA method, if SST6 streaking with 27 zigzags was applied. WASP SST2
280 streaking using 11 zigzags was significantly inferior in generating single colonies. Thus,
281 the critical parameter was the streaking pattern selected, i.e. number of zigzags, rather
282 than the instrument used for automated inoculation. Differences in streaking patterns
283 may be the reason for discrepancies between the results of this study and the results of
284 the studies of Croxatto, Froment, and Mischnik as discussed above (4, 5, 13).

285 Both WASP SST6 and InoqulaA were superior to manual streaking in maximizing
286 single colony recovery. These findings are in agreement with those of other authors (4).

287 The same study found a significant improvement of automation over manual processing
288 mainly for high CFU counts ($>10^6$ /ml), the present study demonstrates a significant
289 increase in recovery of single colonies using automated inoculation for CFU counts
290 down to 10^4 CFU/ml (4).

291 ***Head to head comparison of clinical samples***

292 WASP processing of clinical specimens yielded significantly more morphologies
293 and species recovered as compared to manual processing, independently from the
294 number of CFU counts present in the sample. This finding parallels those of a previous
295 study that analyzed urine specimens processed with the BD-Kiestra system versus
296 manual handling (5, 18). Indeed, next to a higher number of morphologies and species
297 isolated, automated processing by WASP lead to detection of more potential pathogens.
298 Other authors evaluating the BD-Kiestra system with urine specimens did not find more
299 pathogens; however, plate reading was not done by camera but by the unaided eye
300 (18). The high resolution of the WASPLab imaging system may essentially contribute to
301 the higher number of morphologies detected, resulting in a higher number of identified
302 species and pathogens.

303 The percentage of categorization of urine samples in clinical reports as “positive”,
304 i.e. treatment indicated, or “negative”, i.e. no treatment indicated, did differ but not
305 statistically significant between automated or manual handling. These results are in
306 agreement with data of other studies evaluating the BD-Kiestra system (18).

307 The results of the head to head comparison of clinical samples demonstrated
308 that automation in urine microbiology is feasible. However, despite a higher number of
309 potential pathogens within “positive” samples in the automated workup, the overall

310 categorization of samples as positive or negative was not significantly different between
311 automated and manual processing.

312 A limitation of this study is that a reliable evaluation of the time to result was not
313 possible, because administrative and organizational lab procedures (for instance no
314 24h/7d service) must be adapted during the process of automated workflow
315 implementation as the influence of these factors on time to result is significant. As the
316 system was primarily evaluated technically, and WASPLab was not part of the
317 diagnostic routine workflow yet, reliable conclusions regarding time to result could not
318 be drawn from this study and should be subject to a follow up work. There are three
319 main conclusions that can be drawn from the present study: i) The streaking pattern, in
320 particular the number of zigzags/length of streaking lines is of critical importance for
321 increasing the number of single colonies recovered from primary cultures; ii) Automated
322 streaking by WASP can significantly increase the number of single colonies recovered,
323 iii) Automated WASPLab processing results in a higher number of detected
324 morphologies, species, and pathogens. In summary, our findings demonstrate that
325 automated sample processing by WASPLab is going to improve the quality of urine
326 microbiological analysis.

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392 **Acknowledgements**

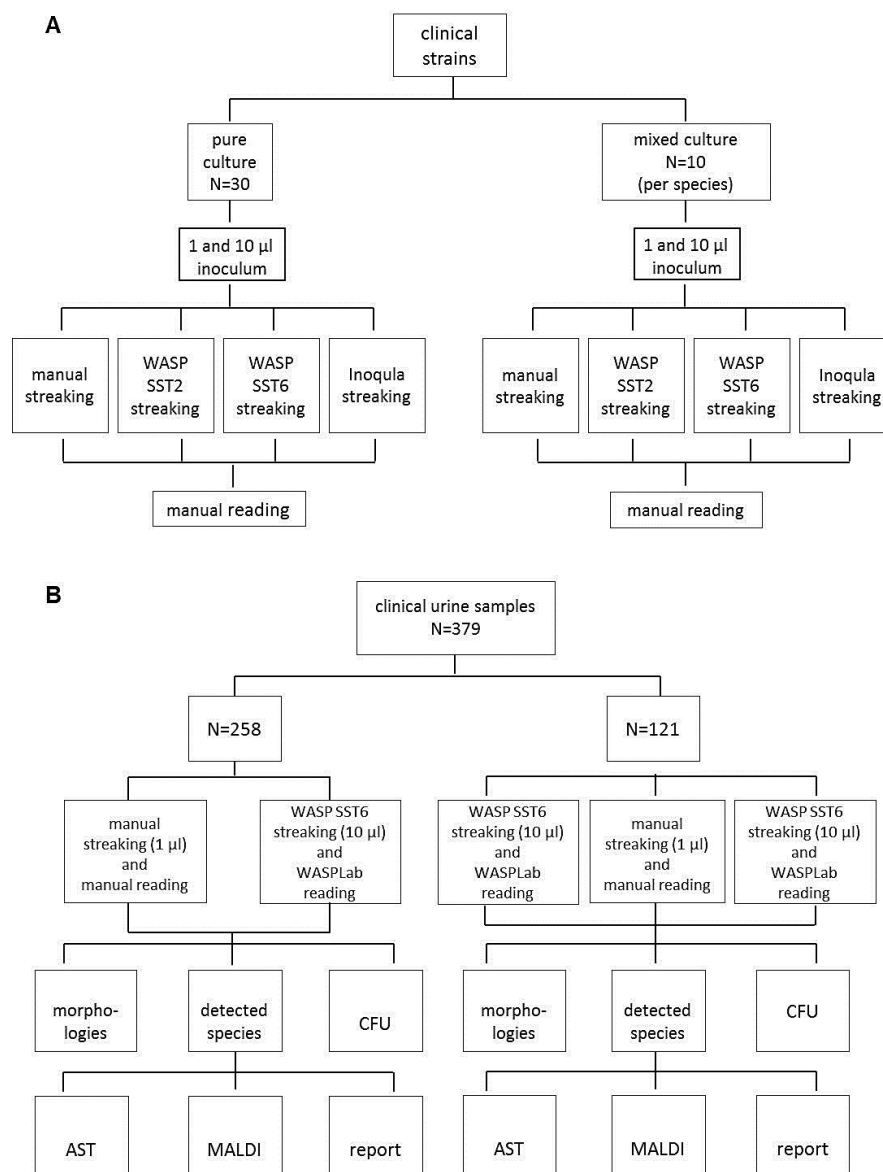
393 This study was partially supported by the University of Zurich.

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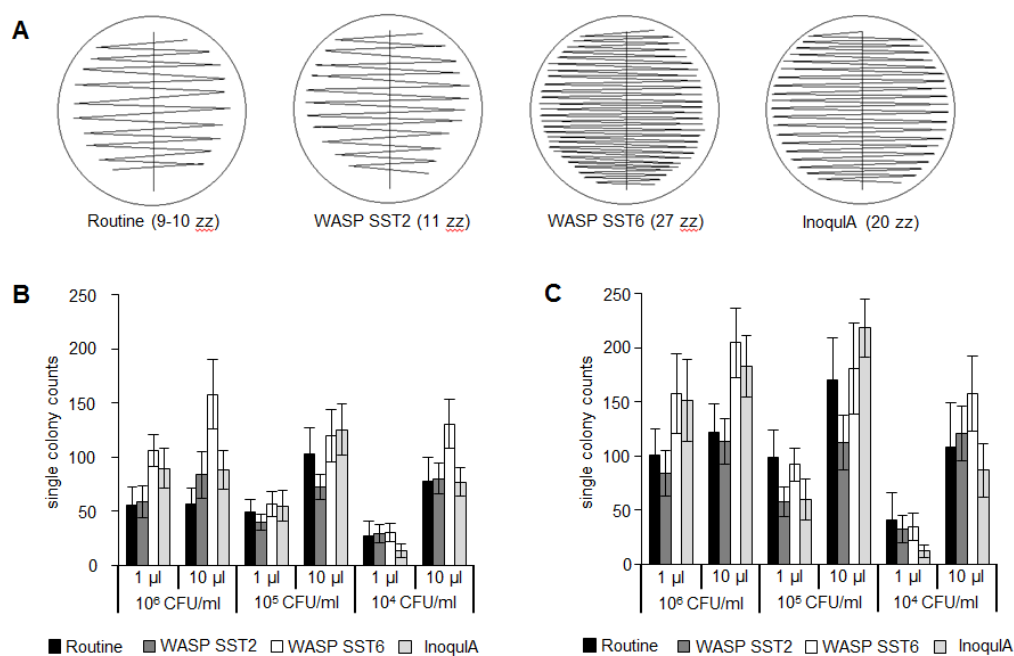
396 **Figures**

397 **Figure 1.** Study design and evaluation steps of part 1 the evaluation of the optimal
 398 inoculation volume and streaking pattern (A) and part 2 the head to head comparison
 399 (B).



400

401 **Figure 2.** Streaking pattern details and resulting numbers of single colony counts. (A)
 402 One manual (routine) and three automated quantitative streaking patterns with varying
 403 numbers of zigzag (zz) lines shown in brackets; WASP single streak type 2 (SST2);
 404 WASP single streak type 6 (SST6) and InoquiA. Comparison of single colonies obtained
 405 by manual and automated streaking. 1 μ l and 10 μ l *E. coli* (B) and enterococci (C)
 406 bacterial suspensions were used with CUFs ranging from 10^4 to 10^6 . The mean values
 407 of 30 clinical strains per species are shown with their standard deviation.



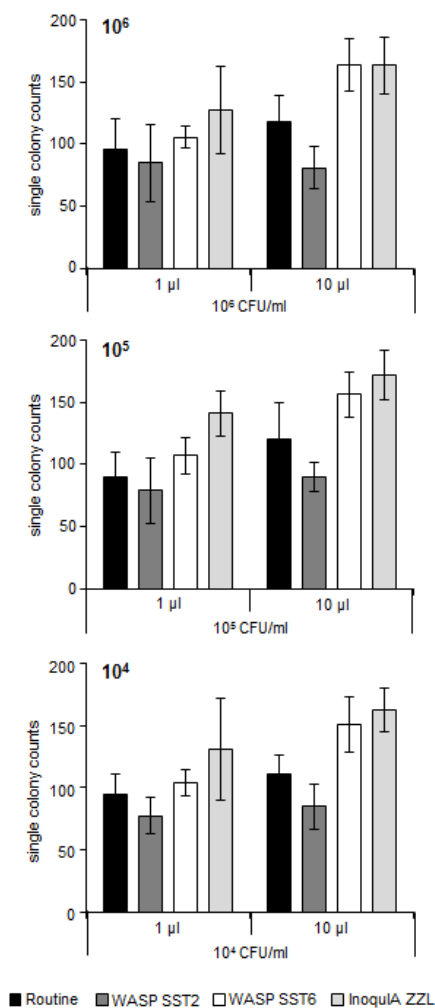
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412 **Figure 3** Cumulated numbers of single colonies recovered from mixed cultures
413 containing variable CFU counts of *E. coli* (10^4 to 10^6 per ml as indicated in the Figure)
414 PLUS fixed CFU counts of 10^6 per ml of both enterococci and coagulase negative
415 staphylococci. Mean values of ten mixed cultures prepared with individual clinical
416 strains are shown with the according standard deviation.



417

418 **Figure 4** Correlation of morphologies (A), recovered species (B), and CFU resulting (C)
 419 from the head to head comparison of 121 and 379 clinical samples, respectively. Data
 420 indicate a shift to higher numbers for all three parameters for automated as compared to
 421 manual inoculation.
 422

A Morphology Correlation

WASP 1 μ l	0	1	2	3	4	≥ 5
Manual						
0	37	1	1			
1	1	12	11	1		
2			5	4	2	4
3			3	9	6	3
4				1	5	9
≥ 5					3	3

WASP 10 μ l	0	1	2	3	4	≥ 5
Manual						
0	73	29	10	2		
1		30	30	8		1
2		1	18	18	13	6
3		1	5	24	29	13
4				6	19	21
≥ 5				2	7	13

B Recovered species correlation

WASP 1 μ l	0	1	2	3	4	≥ 5
Manual						
0	55	8	4			
1	1	33	6			
2		5	6			
3		1	1	1		
4						
≥ 5						

WASP 10 μ l	0	1	2	3	4	≥ 5
Manual						
0	161	35	5			
1	8	91	24	1		
2	4	9	30		1	
3		1	3	5		
4						
≥ 5				1		

C CFU correlation

WASP 1 μ l	n. g.	< 10 ⁴	10 ⁴	10 ⁵	10 ⁶	$\geq 10^6$
Manual						
n. g.	37	2				
< 10 ⁴	1	17	8		1	
10 ⁴		4	14	2		
10 ⁵			1	12	4	
10 ⁶				3	15	
$\geq 10^6$						

WASP 10 μ l	n. g.	< 10 ⁴	10 ⁴	10 ⁵	10 ⁶	$\geq 10^6$
Manual						
n. g.	73	40	1			
< 10 ⁴		74	18	6	1	
10 ⁴		11	30	19	7	
10 ⁵		2	1	22	30	
10 ⁶				4	40	
$\geq 10^6$						

423

424 **Table 1.** Results of the head to head comparison of clinical samples. Manual inoculation
 425 versus WASP inoculation (10 µl loop). Significant differences are indicated in bold (non-
 426 parametric Wilcoxon signed rank test ($p < 0.05$) and crosstab chi-square test of
 427 independence ($p < 0.05$).

428
 429

Parameter	N Samples with indicated parameter (N, % of all samples)					p
	Manual	WASP	Identical	Manual > WASP	WASP > Manual	
Total number of samples	379					
Morphologies			177 (46.7%)	22 (5.8%)	180 (47.5%)	0.000
Colony forming units (CFU) /ml			239 (63.1%)	14 (4.8%)	122 (32.2%)	0.000
Recovered species			287 (75.7%)	26 (6.9%)	66 (17.4%)	0.000
Total No. of MALDI-TOF identifications	253	313				0.000
Total No. of susceptibility tests	149	163				0.337
Report			199 (52.5%)		180 (47.5%)	
Positive result	141 (37.2%)	153 (40.4%)				
Negative result	238 (62.8%)	226 (59.6%)				
Possible Pathogens¹⁾	159 (42%)	172 (45.4%)				
Contaminated	10 (2.6%)	10 (2.6%)				

430
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 433

¹⁾ Possible Pathogens: Gram negative rods, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Staphylococcus saprophyticus*, *Streptococcus group B, C or G*, enterococci, yeasts, *Corynebacterium glucuronolyticum*, *Corynebacterium urealyticum*

434 **Table 2.** Results of the head to head comparison of clinical samples. Manual inoculation
 435 versus WASP inoculation (1 µl loop). Significant differences are indicated in bold
 436 (non-parametric Wilcoxon signed rank test (p<0.05) and crosstab chi-square test of
 437 independence (p<0.05).
 438

Parameter	N Samples with indicated parameter (N, % of all samples)					p
	Manual	WASP	Identical	Manual > WASP	WASP > Manual	
Total number of samples	121					
Morphologies			71 (58.7%)	8 (6.6%)	42 (34.7%)	0.000
Colony forming units (CFU) /ml			95 (78.5%)	9 (7.4%)	17 (14.1%)	0.176
Recovered species			95 (78.5%)	8 (6.6%)	18 (14.9%)	0.043
Total No. of MALDI-TOF identifications	76	100				0.001
Total No. of susceptibility tests	47	52				0.601
Report			79 (65.3%)		42 (34.7%)	
Positive result	47 (39.7%)	51(42.1%)				
Negative result	73 (60.3%)	70 (57.9%)				
Possible pathogens ¹⁾	48 (39.7%)	59 (48.8%)				
Contaminated	6 (5%)	2 (1.7%)				

439

440

441 ¹⁾ Possible Pathogens: Gram negative rods, *Staphylococcus aureus*, *Staphylococcus lugdunensis*,
 442 *Staphylococcus saprophyticus*, *Streptococcus group B, C or G*, enterococci,
 443 yeasts, *Corynebacterium glucuronolyticum*, *Corynebacterium urealyticum*
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