Processing of positive blood cultures using total laboratory automation – chances, challenges and limits

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Introduction

Laboratory automation entails a substantial reorganization of the classic manual microbiology workflow. For screening and urine samples automation resulted in a reduced time to report and an increase in detection of gram-positive bacteria. Automation of blood cultures is challenging due to identical lab numbers for paired blood culture bottles, generation of gram slides and gram-morphology driven initial susceptibility testing. Additionally, processing blood cultures is already a highly optimised workflow in the manual setting.

Materials and Methods

We integrated our blood culture workflow into an existing automation system, which already processed up to 600 samples per day. We implemented a workflow incorporating the creation of gram slides and subculture plates for each bottle type. In order to work with the FA-module of the InoculA (BD Kiestra) gram-slide preparation and subcultures were done from a NaCl (0.85%) dilution of the positive blood culture fluid. Results of gram-slides were compared. Different imaging times and inoculation volumes for plates were assessed for suitability. We compared/analyzed the data of more than 200 bottles of true blood cultures (aerobic and anaerobic bottles) processed either manually or with the automated workflow.

Results

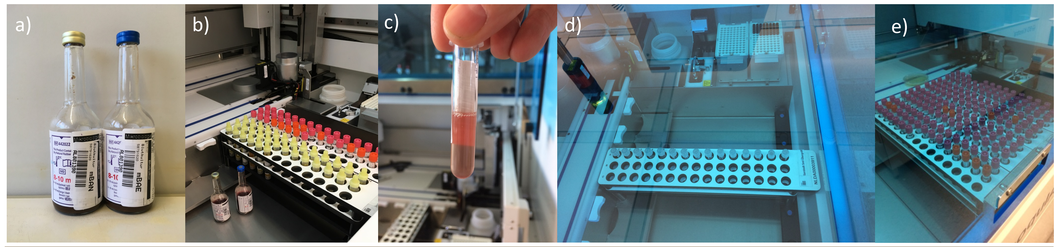

Figure 1: Obstacles: a) identical lab numbers on aerobic and anaerobic bottle; b) InoculA can not draw liquid directly from blood culture bottle; c) + d) solution: 2.5ml NaCl (0.85%) tube containing 3-5 drops of liquid from the positive blood culture bottle; e) fast processing of individual positive blood cultures?

	Blood agar (10µl)				Chocolate agar (10µl)				Blood agar (100µl)			
	5h	6h	7h	24h	5h	6h	7h	24h	5h	6h	7h	24h
Aerobic Bottle												
No growth	21	16	11	3	20	16	10	4	9	7	4	2
Minimal growth	24	7	8	0	26	10	9	0	6	3	6	0
growth	**39**	**61**	**65**	**81**	**38**	**58**	**65**	**80**	**19**	**24**	**24**	**32**
Total aerobic	84	84	84	84	84	84	84	84	34	34	34	34
Anaerobic bottle												
No growth	11	7	5	3	13	8	8	3	2	1	1	0
Minimal growth	32	9	2	0	27	11	6	0	11	2	0	0
growth	**31**	**56**	**65**	**69**	**32**	**53**	**58**	**69**	**21**	**31**	**33**	**34**
Total anaerobic	72	72	72	72	72	72	72	72	34	34	34	34
SUM	**156**	**156**	**156**	**156**	**156**	**156**	**156**	**156**	**68**	**68**	**68**	**68**

Table 2: growth kinetics of positive blood culture samples on different media

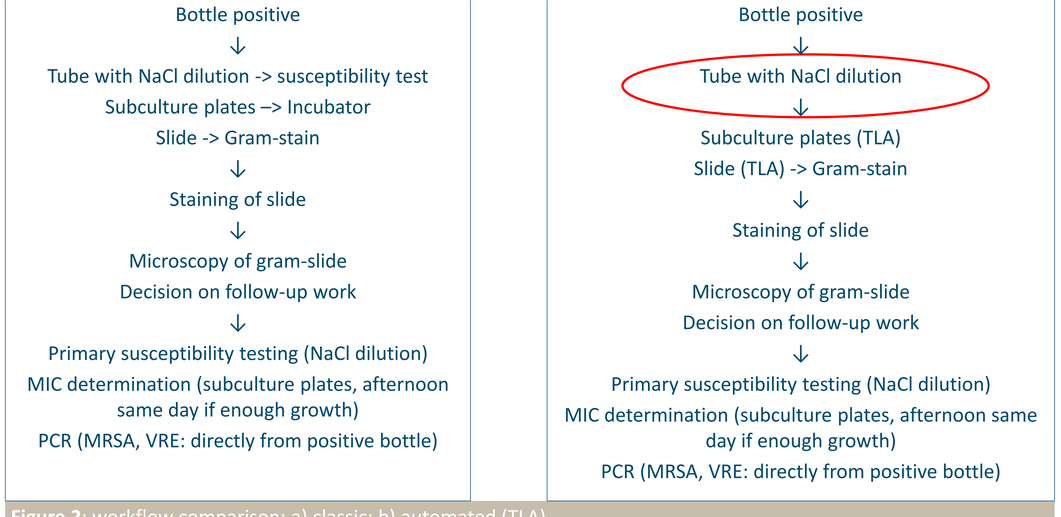

Figure 2: workflow comparison: a) classic; b) automated (TLA)

a) Gram-slide: identical result

Morphology		No.	
cocci	uncharact.	4	
	pairs	6	
	clusters	98	
	chains	27	135 (64%)
rods	Gram-neg.	59	
	Gram-pos.	3	
	Gram-lab.	1	63 (30%)
yeasts		9	9 (4%)
No microorg.		5	5 (2%)
SUM		**212**	**212**

b) Gram-slide: mismatch - analysis

Mismatch	No.	Morphology	Culture
Cocci morph mismatch	7		
Rod morph mismatch	4		
No microorg. TLA	8 (3,5%)	Morphology classic 2 x cluster 2x gram-neg. rod 2x Propioni 2x yeast	Culture *S. aureus, S. epidermidis* *E. coli*, Pasteurella Propioni *C. albicans, C. glabrata*
No microorg. Classic	4 (1,7%)	Morphology TLA 1x cluster 2x gram-positive rod 1x yeast	Culture *S. haemolyticus* Contamination NaCl *C. albicans*
SUM	**23**		

Table 1: Comparison of gram-slides from classical workflow to TLA workflow: a) 212 of 235 (90%) were identical ; b) mismatches (10%); c) gram-slides prepared by the TLA;

Conclusions

1. The development of an automated workflow for blood cultures is more complex than for urine or screening samples.
2. The main limitation for workflow acceleration is the growth kinetic of bacteria on plates.
3. The integration of blood cultures into automation has the advantage of giving standardized results.
4. It is well feasible for en-bloc samples in the morning.
5. It is difficult for singular positive samples during daytime.