Dilution of positive blood culture samples does not impact the microscopic examination

Giulia Gatti[1], Francesca Taddei[2], Maria Sofia Montanari[2], Anna Marzucco[2], Giorgio Dirani[2], Silvia Zannoli[2], Martina Brandolini[3], Laura Grumiro[2], Alessandra Mistral De Pascali[3], Alessandra Scagliarini[3], Vittorio Sambri[2,3], Monica Cricca[2,3]

1: Department of Industrial Engineering - DIN, Alma Mater Studiorum-University of Bologna

2: Department of Medical and Surgical Sciences -DIMEC, Alma Mater Studiorum-University of Bologna

3: Unit of Microbiology, The Great Romagna Hub Laboratory

Background

Sepsis is a complex clinical setting, and its treatment requires a prompt and precise diagnostic intervention. Blood Cultures (BC) slides smear interpretation is used, in some cases, as a useful tool for presumptive identification and laboratory automation can speed up the diagnosis. Copan introduced a workflow for automatic processing of BC for Rapid AST (RAST) using Radian® (Copan) that requires the BC 1:3 dilution to inoculate RAST plates. This proof-of-concept study assesses the impact of BC dilution of on microscopic examination.

Materials and Methods

One-hundred sixteen (116) leftover BCs bottles, with a time-to-positivity higher than 20 hours, were collected from two-months diagnostic routine; 107 positive samples (52 bacteria and 55 fungi) and 9 negatives were included. For each sample, an aliquot of BC bottles was transferred in an empty tube to obtain the undiluted sample (*tq*). A second aliquot was transferred in a tube pre-filled with saline to obtain the 1:3 diluted sample (*dil*). *tq* and *dil* samples were smeared on microscope slides. Slides were stained with Gram reagents, randomized, and blindly read under the microscope by a second operator (B). Simultaneously samples were inoculated on Columbia agar + 5% sheep blood (COS) plates and incubated overnight to check the quality of microbial growth. MALDI TOF-MS identification was also reported for additional reference.

A comparative assessment of the morphological and Gram characterization of organisms in *tq* and *dil* samples was made following the questionnaire filled in by operator B, including the time to complete the analysis.

Results

Agreement between *tq* and *dil* slides regarding the microscopic evaluation was equal to 100%.

The reading time was equivalent for both tq (average 2.18') and dil (average 1.88'); the microbial growth on COS was comparable in terms of organism recovery.

Microscopic interpretation		Undiluted sample (*tq*)							
		Streptococci	*Staphylococci*	Yeasts	Gram-neg bacilli	Gram-pos bacilli	Gram-pos cocci	Other	Negative
Diluted sample (*dil*)	*Streptococci*	8							
	Staphylococci		22*						
	Yeasts			56*					
	Gram-neg bacilli				18				
	Gram-pos bacilli					1			
	Gram-pos cocci						2		
	Other							2	
	Negative								9

* Two samples displayed both *Staphylococci* and yeasts

Conclusions

The introduction of the 1:3 dilution does not affect the quality of the preliminary identification of bacteria and fungi on Gram-stained slide in comparison with the undiluted sample. The results indicate that, in the automatization context, the diluted sample intended for automated Rapid AST can be used simultaneously for slide preparation, maintaining optimal turnaround time and minimizing handling effort.

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