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Manual versus automated plate streaking of stool samples: a comparative evaluation using PREVI Isola®

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Background

A recent problem of many microbiology laboratories is the annually rising number of samples and requested analyses. Due to economic necessities the staff capacity is limited and in many labs is actually declining.

The processing of specimens, especially the plating, is a manual and time-consuming step in lab work flow. Attempting to gain a higher efficiency in this step we evaluated an automated instrument for inoculating and spreading urine samples onto agar plates 13 month ago. As one of the first labs we used the PREVI Isola® (Biomerieux, France) directly connected to our LIS for this purpose. Within this time period we streaked about 90.000 plates on the instrument with a failure rate oscillating near 1%.

To increase the utilization of the streaker in our lab we started to plate stool samples in August 2009 when the instrument was licensed for this samples.

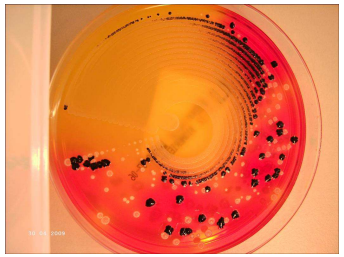

Fig.1 Fecal sample with Salmonella Enteritidis streaked by PREVI Isola® on XLD agar. Salmonella colonies showing a typical black morphology.

Material and Methods

Fecal samples were either streaked by the manual loop-to-plate method (10 µl) or by the PREVI® Isola streaker. The same set of culture media was used:

Columbia blood agar, XLD agar (Salmonella, Shigella), Yersinia agar (CIN) and Campylobacter agar.

As only liquid samples can be processed with the streaker, 1 g of each fecal sample was suspended in 1.5 ml saline (0.45% NaCl) and 18 µl of this suspension were plated.

In addition an aliquot of each sample was added to selenite enrichment broth, incubated for 18 hours and streaked on Leifson agar.

To evaluate the sensitivity of the PREVI streaker serial dilutions of enteric pathogens (Salmonella, Shigella, Yersinia and Campylobacter) were spiked into fecal samples and plated with both methods. Colonies were counted after 24 and 48 hours.
The technical staff judged the quality of the automated streaking using criteria as morphology of colonies, number of isolated colonies per plate and their personal impression as superior, equal or inferior to the manual method. Each streaking was judged by 3 technicians independently.
In a retrospect analysis the detection rate of enteric pathogens in all stool samples plated manually between February and July 2009 (6 month) was compared to the findings in automated streaked fecal samples between August 2009 and January 2010.

		48 hours	
	(CFU/ml)	PREVI®	manual
Salmonella Enteritidis	1.5 x 10e5	+++	+++
	3.0 x 10e4	++	++
	6.0 x 10e3	+	+
	1.3 x 10e3	+	+
Shigella boydii	1.5 x 10e5	+++	+++
	3.0 x 10e4	++	++
	6.0 x 10e3	+	+
	1.3 x 10e3	+	+
Yersinia enterocolitica	1.5 x 10e5	++	++
	3.0 x 10e4	+	+
	6.0 x 10e3	+	+
	1.3 x 10e3	+	+
Campylobacter jejuni	1.5 x 10e5	++	++
	3.0 x 10e4	+++	++
	6.0 x 10e3	++	+
	1.3 x 10e3	+	+

Tab.1 Serial dilution of enteric pathogens in fecal samples resulted in comparable recovery rates with both methods.

Results

Serial dilutions of the main enteric pathogens Salmonella, Shigella, Yersinia and Campylobacter showed comparable detection rates on specific culture media either plated manually or by automated streaking (Tab. 1). No loss of sensitivity could be detected after suspension of 1 g fecal sample in 1.5 ml saline compared to direct plating of stool.

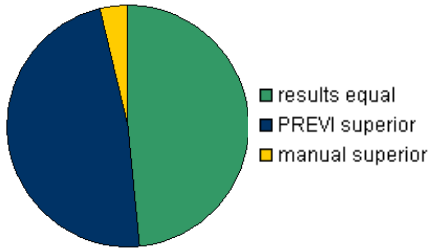

Fig.2 Feasibility of automated plate streaking in the judgement of the technologists showed non-inferiority in more than 95% of the samples.

Results

The suitability of the automated streaking was judged by the technical staff as superior to manual plating in 48% and as equally in another 48%. Only in 4% a disadvantage was documented by the technicians. So feasibility of the automated streaking clearly shows non-inferiority (Fig. 2).

Preliminary data showed a faster processing of plating fecal samples, when using PREVI® Isola. The technologist's hands-on time is reduced by 46% or nearly 5 minutes for plating 15 samples (Fig. 3).

Over 6 month each the detection rates of enteric pathogens were compared in a retrospect analysis between manually plating and automated streaking in more than 4000 fecal samples. While comparable low numbers of Shigella spp. were found, a 50% increase in Salmonella frequency (1.2 up to 1.8%) occurred. Especially a 3-fold higher number of Campylobacter strains were isolated from the streaked stool aliquots, although normally a higher detection rate for this species is found between May and July. No outbreaks were reported within this time period in our region.

	PREVI Isola	Manual Method
Average time to perform 15 fecal samples	5:29 min.	10:17 min.
Average time to perform 1 fecal sample	21,9 sec.	41,1 sec.
Time difference to perform 15 fecal samples	**4:48 min.**	

Fig. 3 Reduced hands-on time for automated plating of stool samples compared to manual methods.

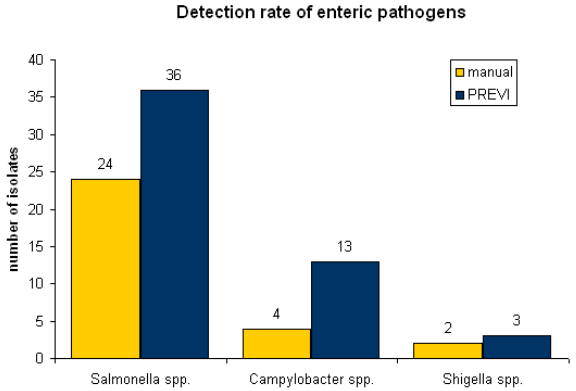

Fig.4 Detection rates of typical enteric pathogens comparing 6 month of manual streaking (n = 2029) with 6 month of automated streaking (n = 1972)

Discussion

We investigated the use of the PREVI® Isola streaker in a comparative evaluation for the automated plating of fecal samples.

The recovery of plated enteric pathogens was comparable to manual methods. The streaker showed a high reliability and stability. We never detected any cross-contamination between different samples.

The subjective evaluation by the technical staff resulted in a positive acceptance. The hands-on time for plating fecal samples was reduced by more than 40%. Despite this fact investment costs and consumables are not completely covered by this retrenchment. Therefore an increase in quality is necessary in addition.

Surprisingly we found a 50% higher detection rate for Salmonella and a threefold rise in the Campylobacter frequency (0.2 to 0.7%). As we offer our diagnostic service mainly to a tertiary care hospital our detection rates might be lower than in laboratories serving primary healthcare facilities.

Microbiology laboratories are being required to manage increasing demands for service under a strong pressure for cost containment. A technology that allows automation of laborious tasks (like labeling, inoculation and streaking) will provide technicians with time to concentrate on higher-level activities.

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