



Comparison of Time to Positivity of the VersaTREK® REDOX 80 ml and the REDOX EZ Draw 40 ml Blood Culture Bottles for Common Bacterial Bloodstream Pathogens

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Abstract

The VersaTREK® microbial detection system (Trek Diagnostic Systems, Cleveland OH) offers two media formulations (an aerobic and an anaerobic bottle) available in two bottle designs; a 40 ml direct draw format and an 80 ml format. The 40 ml EZ Draw® (EZD) bottle can be inoculated with a maximum volume of 5 ml while the 80 ml bottle accommodates a 10 ml maximum fill. It is widely reported in the literature that blood volume drives recovery of microbial pathogens. However, the effect of blood volume on time to positivity (TTP) has not been clearly established with these bottle types. This study utilized seeded samples to evaluate the impact of blood inoculum volume and by consequence organism concentration on TTP for the two bottle types.

REDOX 80 ml and EZD bottles were inoculated with 8 ml and 4 ml of blood respectively from healthy donors. The bottles were then seeded with standardized inocula to mimic microbial bloodstream concentration during bacteremia. EZD bottles were inoculated with half the concentration of organism seeded into the 80 ml bottles. Bottles were mixed and loaded onto the Trek instrument for 5 days of incubation as per manufacturer's instructions. The bacterial strains utilized included common aerobic and anaerobic bloodstream pathogens. Each organism was tested in triplicate and concentration of organism per bottle was determined by plating inocula in triplicate. TTP was determined using the first bottle to signal positive in each set regardless of media type (aerobic or anaerobic).

Concentration of inocula in the 80 ml bottles ranged from 9 to 134 colony forming units per bottle. All organisms tested were detected by either one or both bottles in every set. Overall, with some exceptions, in spite of the lower inocula concentration, organisms tended to flag positive earlier on the EZD bottles although this difference in TTP was not statistically significant ($p < 0.01$). The data generated in this study using simulated blood cultures indicated that the difference in volume of blood inoculated did not significantly impact TTP in the EZD bottle as compared to the REDOX 80 ml bottle.

Background

- TTP is dependent on the bacterial load in the blood and can play an important role in clinical decision making and interpretation of positive blood cultures
- Differential TTP for paired blood cultures is useful for detection of catheter related blood stream infections. In *Staphylococcus aureus* bacteremia, it may also identify presence of endovascular source, metastatic lesions, attributable mortality and other complications (1).
- TTP has also been found to be a predictor of mortality in patients with *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae* bacteremia and septic shock.(2,3,4)
- Previous studies have shown that in the majority of cultures tested, growth is detected earlier using 10 ml vs 5 ml of blood (5). However these studies compared different volumes in the same bottle type.
- The EZD bottle can be inoculated with a maximum of 5 ml of blood which is half the maximum volume that can be inoculated into the REDOX 80ml bottles (10 ml).
- We utilized EZD and REDOX 80ml bottles inoculated with blood from healthy donors that had been seeded with target organism at known concentrations to determine the impact of difference in blood volume on TTP.

Materials and Methods

- Whole blood from healthy donors acquired from the American Red Cross was spiked with ATCC and laboratory characterized bacterial isolates. Paired aerobic and anaerobic REDOX 80 ml and EZD bottles were inoculated with 8 ml and 4 ml of blood respectively. Blood culture sets were then seeded in triplicate with target organism at concentrations of 1-100 cfu/bottle to represent levels of bacteremia during septic episodes. The EZD bottles received half the volume of inocula as compared to the REDOX 80 ml bottles to mimic the effect of reduced sample volume on organism load. Inocula was plated in triplicate to appropriate media to determine concentration. Cultures were then processed as per manufacturer's instructions. Bottles flagging positive were removed from the instrument, TTP was recorded, bottle was also Gram stained and subcultured to confirm the growth of target organism. Difference in mean TTP between REDOX 80 ml and EZD bottles was calculated. Statistical significance was determined using student *t* test.

Results

Organism	REDOX 80 ml			EZD			Difference (h)
	TTP (h)	SD (h)	cfu/bottle	TTP (h)	SD (h)	cfu/bottle	
<i>Escherichia coli</i>	12.3	0.1	50.2	11.7	0.3	25.1	0.6
<i>Klebsiella pneumoniae</i>	11.0	0.1	43.2	10.9	0.3	21.6	0.0
<i>Enterobacter cloacae</i>	12.0	0.2	71.8	11.9	0.2	35.9	0.1
<i>Proteus mirabilis</i>	10.5	0.0	56.7	10.8	0.4	28.3	-0.3
<i>Staphylococcus aureus</i>	12.6	0.4	72.3	11.9	0.0	36.2	0.7
<i>Staphylococcus epidermidis</i>	16.1	0.5	31.0	16.0	0.5	15.5	0.2
<i>Streptococcus pyogenes</i>	11.8	0.1	48.7	11.5	0.3	24.3	0.3
<i>Streptococcus agalactiae</i>	14.9	0.5	52.7	14.1	0.9	26.3	0.9
<i>Streptococcus pneumoniae</i>	16.0	1.6	8.7	14.3	0.7	4.3	1.7
<i>Enterococcus faecium</i>	13.2	0.1	30.2	12.8	0.2	15.1	0.4
<i>Streptococcus bovis</i>	12.5	0.3	122.0	12.2	0.3	61.0	0.3
<i>Pseudomonas aeruginosa</i>	14.9	0.1	79.2	15.1	0.2	39.6	-0.2
<i>Haemophilus influenzae</i>	16.6	1.4	30.3	14.7	0.8	15.2	1.9
<i>Campylobacter jejunii</i>	36.4	1.0	15.5	34.0	0.8	7.8	2.5
<i>Bacteroides fragilis</i>	25.7	0.4	134.0	24.2	1.1	67.0	1.5

Table 1: Mean TTP and concentration of inocula for the REDOX 80 ml and EZD blood culture bottles for target organisms. TTP for each culture set is the time taken for any bottle in a set to flag positive whether aerobic or anaerobic. Difference = (TTP for REDOX 80 ml) – (TTP for EZD).

Results and Discussion

•Even at very low inocula concentrations, both REDOX 80 ml and EZD bottles consistently detected all organisms tested in at least one bottle in every paired culture set.

•In spite of the difference in inocula, there was no statistically significant difference ($p < 0.01$) in TTP between REDOX 80 ml bottles and EZD bottles for the organisms tested.

•Mean TTP of *Enterobacteriaceae* for the REDOX 80 ml and EZD bottles was 11.4 and 11.3 h respectively.

•Mean TTP of Gram positive cocci for the REDOX 80 ml and EZD bottles was 13.9 and 13.2 h respectively.

•For 13/15 organisms tested, the EZD bottle flagged positive earlier than the REDOX 80 ml bottle despite of the lower concentration of inocula. This difference however was not statistically significant ($p < 0.01$).

•It can be hypothesized that this observation may be due to the fact that the VersaTrek instrument depends on detection of changes in headspace pressure to flag bottles positive for growth. The trend towards lesser TTP with the EZD bottles may be due to its smaller headspace as compared to the REDOX 80 ml bottle.

Conclusions

There is no statistically significant difference in TTP between the REDOX 80 ml and EZD blood culture bottles using whole blood spiked with selected organisms at known concentrations.

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