Validation of manual blood culture bottles V.2

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Use of equipment-free, “manual” blood cultures is still widespread in low-resource settings, as requirements for implementation of automated systems are often not met. Quality of manual blood culture bottles currently on the market, however, is usually unknown. An acceptable quality in terms of yield and speed of growth can be ensured by evaluating the bottles using simulated blood cultures. In these experiments, bottles from different systems are inoculated in parallel with blood and a known quantity of bacteria. Based on literature review and personal experiences, we propose a short and practical protocol for an efficient evaluation of manual blood culture bottles, aimed at research or reference laboratories in low-resource settings. This laboratory protocol was used in a study for Médecins Sans Frontières' Mini-Lab project, which aims to bring clinical bacteriology to low-resource settings. Three bottle types were evaluated in this study; two "manual" blood culture bottles and one automated system.

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This protocol describes the steps needed to evaluate (manual) blood culture bottles using simulated blood cultures. We added "Materials" to the previous version of the protocol.

blood culture; in vitro evaluation; blood culture bottles; manual blood culture systems

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Devices:
- vortex
- densitometer
- incubator
- roller-mixer (optional)
- 100 µL - 1000 µL pipettes
- pipet controller (for 10 mL glass pipettes)

Consumables:
- human or horse blood
- sterile saline
- 50 mL tubes
- 10 mL tubes
- 10 µL loops
- 10 mL syringes
- blood culture bottles
- 10 mL glass pipettes
- 300 µL pipette tips
- Columbia agar 5% sheep blood

Spiking of the blood (human or horse)

1 Spiking of the blood (human or horse) is done with frozen (-80°C) clinical or ATCC strains

2 Strains to be tested are taken from the -80°C freezer (conservation on microbeads) and plated out on blood agar. The plates are incubated for 18 – 24 hours at 35°C. When necessary (*e.g.* fastidious organisms), incubation under CO_2 atmosphere is done. A second inoculation is done from these colonies on blood or chocolate agar under the same conditions (two passages). It is not advised to keep the plates in the incubator or fridge for more than 36 hours, as some more vulnerable bacteria die on the plates in our experience (especially *B. cepacia*).

3 The blood is homogenized by using a roller-mixer (optional)

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4 When the agar plates show pure growth of the clinical strain, a dilution series is done. A 0.5 McFarland suspension (1.5 x 10^8 CFU/mL) is made in sterile saline. This suspension is diluted with a factor 4*10^5 using three serial dilutions, resulting in a final concentration of approximately 375 CFU/mL. Colony count of this suspension is done by inoculating 100 µL on **three blood agar plates**. The mean of the three colony counts will be taken as final concentration/100 µL.

5 Of this suspension, 1 mL is added to 25 mL of blood for pediatric formulations, resulting in a concentration of approximately **15 CFU/mL of blood**.

6 For each bottle type (bottle under evaluation and automated BCB), three bottles are inoculated with 2 mL of the spiked blood. The number of CFU added is then approximately **30 CFU/bottle**. Number of actual CFU added per bottle can be re-calculated from the plate colony count by multiplying the mean plate colony count with 2/5.

7 For adult formulations, 1 mL of the bacterial end suspension is added to 50 mL of blood, resulting in a concentration of approximately **7 CFU/ml of blood**. The same tube of blood must be used to fill an equal number of reference bottles and bottles under evaluation, to avoid bias.

8 For each bottle type (bottle under evaluation and automated BCB), three bottles are inoculated with 10 ml of the spiked blood. The number of CFU added is then approximately **70 CFU/bottle**. Number of actual CFU added per bottle can be re-calculated from the plate colony count by multiplying the mean plate colony count with 1/5.

9 Manual BCB are incubated in a normal incubator at 35°C. Bottles will be incubated for a total of 7 days. The automated bottles are placed in the automated incubator.

Processing of incubated BCB

10 Twice daily (morning and early afternoon), bottles are taken out of manual incubator and inspected for visual signs of growth

11 Standardized visual conditions are preferable for detection of growth. As normal daylight may show substantial variation, signs of growth are best evaluated using a device spreading diffuse light, *e.g.* use of a lightbox or LED lights. We used a lightbox designed by JP Selecta (Barcelona, Spain).

12 Time and date of visual signs of growth in the broth are noted, and the type of growth is noted:

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o Hemolysis
o Turbidity
o Puff balls
o Pellicle/film
o Gas production

13 For biphasic bottles (if applicable): time and date of visual signs of growth on the agar are noted, and the type of growth is noted:
o Gas production in agar
o Film on agar
o Single colonies
o Confluent growth

14 Bottles are incubated until both broth (and agar, if applicable) show visual signs of growth; or until after 7 nights of incubation (end of incubation period), in case growth is not seen on both agar and/or broth.

15 A blind subculture on blood agar is done for all manual blood cultures 24 hours after inoculation (D1 of incubation, see table 1)

16 Subculture on blood agar of all bottles is done at signs of positivity in broth/agar/automate, if blind subculture did not show colony growth

17 If there are no visual signs of growth on day 7 and/or no growth on any subculture was noted by day 7, a terminal subculture on blood agar is performed

Vacuum testing (optional)

18 10 bottles of each bottle type under evaluation (adult + pediatric if bottles are different) from at least 2 different lots (ideally 3) are tested for the strength of the vacuum present in the BCB

19 Blood cultures sampled with needle and syringe:

19.1 Connect a 21-gauge needle to a 10 ml syringe filled with distilled water

19.2 Perforate the bottle septum and without outside pressure, let the bottle fill

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with water

19.3 The amount of vacuum in the bottle is measured by the amount of water spontaneously aspirated by the bottle

20 Blood cultures sampled with butterfly needle:

20.1 The hub and tube of a 21-gauge butterfly needle is put into a measuring glass of 100 ml filled with distilled water. The amount of water present in the cylinder is marked on the measuring glass.

20.2 Pierce the septum of the bottle with the butterfly needle while the other end is kept stable in the measuring glass.

20.3 After filling of the bottle by the vacuum, the difference between start and stop volume of water is measured and calculated.

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