

## REVIEW

# Blood cultures in newborns and children: optimising an everyday test

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Effective use of blood cultures is a key component of the management of septic newborns and children. The technical and practical aspects of paediatric practice and the heightened susceptibility of children to infection because of immunological immaturity make automatic extrapolation of adult data difficult and potentially unfounded.

**B**lood cultures remain the mainstay of investigation of potential sepsis in infants and children, despite recent advances in the molecular diagnosis of bacterial and fungal sepsis.<sup>1</sup> Most of the evidence for use of blood cultures as an investigation comes from adults. This review discusses the optimal use of blood cultures, and highlights the different challenges posed by newborns, infants, and older children.

The important factors in the ability of blood cultures to detect significant organisms in adults include:

- volume of blood drawn<sup>2-9</sup>
- dilution: the ratio of blood to culture medium in the blood culture bottle<sup>8 10 11</sup>
- number of cultures taken<sup>8 9 12 13</sup>
- blood culture technique, including skin preparation and choice of culture site<sup>14</sup>
- timing of culture<sup>8 9</sup>
- choice of blood culture bottle and system (including whether it preferentially detects aerobic or anaerobic organisms)<sup>12 15 16</sup>

## BLOOD VOLUME

### Neonates

There are few clinical data on the effect of blood volume alone on blood culture outcome in newborns. In the United Kingdom, reported volumes per culture drawn vary from 0.3 ml to 0.66 ml, all well under the lower limit of 1 ml recommended by paediatric blood culture bottle manufacturers.<sup>17-19</sup> On the basis of quantitative blood culture data from the 1970s, with high loads of *Escherichia coli* shown in 80% of infected newborns, it has traditionally been thought unnecessary to draw more than small amounts of blood.<sup>20</sup> Changes in the spectrum of organisms infecting newborns since then, with an increase in Gram positive isolates, particularly coagulase negative staphylococci (CoNS), has prompted investigators to revisit this question.

In a prospective Mexican study in which different blood volumes from the same venepuncture in infants up to 12 months were used,

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“approximately” 2.2 ml blood was drawn from each infant with clinical signs and symptoms of sepsis.<sup>21</sup> Blood was divided into 2.0 ml and 0.2 ml aliquots and injected into culture medium, maintaining a blood to total broth dilution of 10%. Compared with the 2.0 ml aliquot, the 0.2 ml sample was found to have a sensitivity of 95% and specificity of 99% for detection of significant bacteraemia. These results suggest that, whereas maintenance of dilution may be important, blood volume may not be. However, the culture systems used and spectrum of organisms grown differ considerably from those seen in UK centres.

### Paediatric data

A paediatric emergency department study, performed by Isaacman and colleagues,<sup>22</sup> provided another within patient comparison of different blood culture volumes. In a prospective, comparative design, they studied children from 2 months to 18 years (median 15 months) of age presenting with symptoms or signs requiring a blood culture. Up to 11.5 ml blood was drawn from 300 children. Then 10 ml was divided into one 6 ml and two 2 ml aliquots; each was further divided and inoculated into aerobic and anaerobic culture bottles. The remaining 1.5 ml was used in a quantitative culture system. In 30 significant infectious episodes, the 6 ml bleed had greater detection sensitivity at 24 hours than the two 2 ml bleeds combined, and a greater final sensitivity, suggesting the importance of increased blood volume.

An implementation policy aimed at increasing the amount of blood taken from paediatric oncology patients has also shown an increased proportion of blood cultures yielding significant pathogens compared with historical controls.<sup>23</sup>

### Effect of organism density

It is presumed that bacteraemias with high concentrations of organisms require less blood to be sampled than low density bacteraemias.<sup>8</sup> The concentration of a variety of common pathogens in neonatal and paediatric bacteraemias has been documented in numerous studies using quantitative culture systems, listed in table 1.<sup>20 24-29</sup> Despite many organisms occurring in high concentrations, low density bacteraemia is also recorded for most pathogens.

In a laboratory based study, Schelonka and colleagues<sup>30</sup> explored the effect of small blood culture volumes for a variety of common neonatal pathogens. A range of blood volumes containing known concentrations of neonatal pathogens were injected into standard paediatric blood culture bottles. If organisms were present at densities of < 4 colony forming units (cfu)/ml, blood

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**Table 1** Density in blood of neonatal and paediatric pathogens

Organism	Age group	Median cfu/ml	% ≤10 cfu/ml	Total no isolates	Range cfu/ml	Reference
Gram positive bacteria						
Group B streptococci	N	5		2		26
	N/P		44	9		25
	N		47	15		24
<i>Streptococcus pneumoniae</i>	P	51*		9	0.5–200	28
	P		58	12		29
<i>Staphylococcus aureus</i>	N	>100		9		26
	N/P		69	13		25
	N		100	2		24
Coagulase negative staphylococci	N	39.5		25		26
	N/P		12.5	8		25
	N	272*		14	40–4120	27
	N		40	5		24
<i>Enterococcus</i> spp	N	5		4		26
	N/P		75	4		25
	N		100	5		24
Gram negative bacteria						
<i>Escherichia coli</i>	N		23†	35		20
	N/P		68	22		25
	N		54	11		24
<i>Haemophilus influenzae</i> type b	N	8		1		26
	N/P		67	3		25
	N		100	2		24
	P	6293		13	6–24000	28
	P		21	23		29
Yeasts						
<i>Candida albicans</i>	N	26		3		26
<i>Candida parapsilosis</i>	N	18		6		26

\*Mean value.

†&lt;5 cfu/ml.

N, Neonatal data; P, paediatric data; N/P, combined neonatal and paediatric data; cfu, colony forming units.

volumes of 0.5 ml or less had a significantly diminished chance of detecting bacteraemia. This finding did not differ between organisms. Brown and colleagues,<sup>31</sup> however, using similar in vitro techniques, found that placental blood seeded with more than 10 cfu/ml *E coli* or group B streptococcus required only 0.25 ml blood to be consistently detected.

## DILUTION

The natural bactericidal activity of blood reduces the viability of organisms that can be recovered from blood cultures. This is due to innate immunity (including complement, phagocytic white blood cells, and lysozyme), acquired immunity to previously encountered pathogens, and residual antibiotics in the blood. The protective effect of liquid culture media results from dilution of the bactericidal activity by the medium and binding of antibiotics by resins in many media. Medium volumes of blood culture bottles in standard paediatric use vary from 20 to 40 ml.<sup>17</sup> In adult studies, in which blood volumes of 10–20 ml were usually used, dilution appeared to affect culture sensitivity. Maximal sensitivity was reported when the blood volume was 10–20% of the total medium volume.<sup>9–11</sup> This effect appears to be less consistent in paediatric and neonatal studies.<sup>10–32</sup> Kennaugh and colleagues<sup>10</sup> found no effect of dilution between 1:10 and 1:100, using 0.5 ml adult blood seeded with low concentrations of common neonatal and paediatric pathogens.

Different culture systems may produce different sensitivities of detection.<sup>22–33</sup> Some paediatric culture systems warn that with low blood volumes (< 1 ml), species such as *Neisseria* may be vulnerable to the lysing agent, sodium poly-

ethanolamine sulphate, which is present in most culture systems.<sup>17</sup> A final concern of dilution with very small amounts of inoculated blood is that some organisms may require supplementation with additional blood to provide growth factors to enable survival in culture medium.<sup>17</sup> *Haemophilus influenzae* requires NAD and haemin, which are available in blood, as essential cofactors.

## NUMBER OF CULTURES TAKEN

In adults, taking up to three blood cultures per sepsis episode increases the chance of detecting bacteraemia.<sup>8–9, 12–13</sup> There are no neonatal data, as usual practice is to take only one blood culture before starting antibiotic treatment.<sup>34–35</sup> This decreased sampling is attributed to the small circulating blood volumes of neonates, the potential for increased transfusion requirements, the technical difficulties posed, and the possible rapid deterioration of newborns in the setting of sepsis.<sup>36</sup> In children, raising the number of blood cultures to two or three bottles, whether from one or more sites, does increase yield.<sup>22–23, 37</sup>

## BLOOD CULTURE TECHNIQUE: BLOOD VESSEL CHOICE AND SKIN PREPARATION

Peripheral venous or arterial punctures are optimal, with no advantage for arterial cultures.<sup>14</sup> False positive results may occur if sampling is from indwelling vascular devices. These may be colonised with organisms (often CoNS) that are not causing systemic infection. Often an indwelling central vascular line is sampled in addition to peripheral blood cultures, in an attempt to identify central line associated sepsis. The

**Table 2** Periodicity of bacteraemias

Bacteraemia type	Clinical scenario	Reference
Transient	Manipulation of infected tissue	8
	Instrumentation of colonised mucosa	8, 9, 52
Intermittent	Undrained abscesses	8
	Fever and neutropenia	9
	Pneumococcal pneumonia	9
Continuous	Intravascular infection	8

results from one small, within patient, comparative trial of blood cultures in 99 children suggest that culturing from a newly inserted intravenous device does not increase the risk of contamination.<sup>38</sup> There is no necessity to change needles between venepuncture and injecting the blood culture bottles.<sup>39</sup>

Trials assessing skin preparation before culture suggest that alcoholic chlorhexidine, iodine tincture 70% isopropyl alcohol, or a combination of povidone iodine/70% ethyl alcohol are superior to povidone iodine in reducing contamination rates.<sup>38 40 41 41a</sup> Maximal killing of skin organisms does not occur immediately, and it is preferable to wait at least one minute before drawing blood.<sup>9</sup>

### TIMING OF CULTURES

The optimal time to culture for bacteraemia is “as early as possible” in the course of a febrile episode, based on fever following bacteraemia or endotoxaemia within one to two hours.<sup>9</sup> The interval between repeat blood cultures does not appear to be important.<sup>42</sup> There are no neonatal or paediatric data on timing of blood cultures. Table 2 summarises adult data on the periodicity of bacteraemia in a variety of clinical scenarios. The principal effect of timing in neonates is likely to be the low threshold for the start of antibiotic treatment, considerably decreasing the chance of isolating organisms on subsequent cultures.<sup>24</sup> This, coupled with the lack of specificity of signs of neonatal sepsis, compared with children and adults, contributes to the lower rate of significant positive blood cultures in neonates, as blood cultures will be performed for many non-septic episodes.<sup>43</sup>

### CHOICE OF BLOOD CULTURE SYSTEMS

There are a variety of blood culture systems in common use in neonatal units and paediatric hospitals. Most paediatric bottles are optimised for 1–4 ml blood, with limited neonatal comparative data supporting increased sensitivity compared with adult bottles.<sup>44</sup> Differences between systems include medium composition and volumes. Some require “venting” by a needle between the inoculation of blood into the bottle and the bottle indicating a positive result, potentially allowing contaminant organisms into the system. Some centres continue to use adult bottles optimised for 5–10 ml blood, and a few use anaerobic bottles for half of the blood drawn.<sup>45</sup> Blood culture technology has changed from tubes or bottles of liquid

culture medium requiring frequent inspection, microscopy, or blind plating on to solid culture medium to see if growth had occurred to modern, closed, computer based systems, which assess changes in CO<sub>2</sub> indicating growth, every 10–15 minutes.<sup>17 46</sup>

Anaerobic sepsis in the neonatal and paediatric population is now rare, with many centres preferring to use all the blood for aerobic cultures unless specific clinical indications exist.<sup>25 33 47 48</sup> Table 3 lists indications for anaerobic culture bottles to be included.

### INTERPRETATION OF RESULTS

Interpreting positive results depends on clinical presentation, how the culture was taken, the organisms grown, and the time taken for the blood culture to become positive. Some organisms, such as *Neisseria meningitidis* and *Candida albicans*, are nearly always significant, even in the context of a well looking child. Cultures positive with potential pathogens that may also be contaminants are far more difficult to interpret, the most common of which is CoNS. These results must always be interpreted in the specific clinical context in which they are seen. Whereas CoNS grown from a previously well child presenting from the community is almost always a contaminant, CoNS growing three days later from the same child after being in hospital with an indwelling intravascular device may well be significant. There are no highly specific and sensitive criteria for determining the clinical importance of CoNS isolates based on clinical and routine microbiological parameters. Most definitions used in adults and older children involve the same organism being grown from at least two separate bleeds, not taken from indwelling intravascular devices, in a child with clinical features of sepsis.<sup>49</sup> In neonatology, usually only one blood culture is taken before the start of antibiotic treatment, making such definitions difficult to use. Rates of contamination are thought to be highest in neonates.<sup>9</sup> Cultures drawn through indwelling intravenous devices are more likely to be contaminated with CoNS colonising the lumen of the device, which may not be causing systemic infection. Positive blood cultures with higher colony counts and flagging positive within 48 hours of being drawn have been associated with an increased likelihood of significance, but are not absolutely sensitive or specific, and may be affected by prior antibiotic use.<sup>14 50 51</sup>

### CONCLUSION

Simple steps to improve the sensitivity and specificity of blood cultures should remain an achievable cornerstone of everyday neonatal and paediatric practice. These include: proper skin preparation; not culturing through pre-existing intravenous devices without additional peripheral cultures; culturing early in febrile episodes; drawing up to three blood cultures per episode if appropriate; increasing blood volume per culture (at least beyond the newborn period).

Effective use of blood cultures in paediatric practice is a key component of the management of septic newborns and children. The technical and practical aspects of paediatric practice, as much as the heightened susceptibility to infection attributable to immunological immaturity in children, make automatic extrapolation of adult data difficult and potentially

**Table 3** Specific indications for anaerobic blood cultures in newborns and children

- Prolonged rupture of membranes >18 hours or maternal chorioamnionitis
- Poor dentition, severe oral mucositis, or chronic sinusitis
- Abdominal signs or symptoms
- Neutropenic patients receiving high dose steroid therapy (where abdominal signs may be masked<sup>15</sup>)
- Sacral or perianal cellulitis or ulceration
- Patients with human bite wounds or crushing trauma<sup>47</sup>

unfounded. Further research is needed into specific questions about blood cultures in children, such as the effect of blood volume in newborns, and the importance of dilution of very small blood volumes in medium.

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