

Comparison of Two Kinds of Blood Culture Bottles at Random Samples

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Abstract Objective: To compare the detection capability of two kinds of aerobic bottles and anaerobic bottles at clinical random specimens. Method: Selecting 4 clinical departments (Department of Infection, Gastroenterology, Surgery ICU, Dermatology) to make blood cultures used in parallel bottles which have been approved by the state listed domestic aerobic and anaerobic blood culture bottles and BacT / Alert FA and FN ones, French bioMerieux company, in the same blood culture system. Choosing the same patients with two sets of blood culture bottles as qualified samples and analyzing by statistical method. Results: A total of 360 sets of blood culture bottles, each company having 180 sets of blood culture bottles. 31 sets of blood culture bottles show positive, 15 sets of imported bottles, 16 sets of domestic bottles, the positive rate was 8.3%、8.9%. 14 sets of them are positive which are common reported. Defind Imported blood culture as 'gold standard', the true positive rate of the domestic ones was 87.5%, the false negative rate was 12.5%. 263 sets of blood culture bottles were reported as negative, 160 sets of domestic bottles, 163 sets of imported bottles, true negative rate is 96.9%, false positive rate is 3.1%. The detection of bacterial desired average growth time in two manufacturers have no significant difference ($t=0.091$, $P>0.05$). Study of consistency analysis of aerobic bottles and anaerobic bottles in domestic and imported ones shows that the Kappa values of aerobic and anaerobic bottles were 0.6759 and 0.7535. The detection ability of domestic anaerobic blood culture bottle is better than the domestic aerobic ones. Conclusion: Comparing domestic blood culture bottles with the imported ones, positive detection rate, negative detection rate and the average duration of the bacteria detected have no significant differences. Specificity and accordance rate are high, however, at the field of sensitivity and probability of miss, the domestic blood culture bottle has yet to be strengthened. The domestic anaerobic bottles are superior to aerobic ones in the the detection consistency. The detecting capabilities of domestic blood culture bottles and imported blood culture bottles do not differ greatly, but the domestic company still need to strengthen the blood culture quality to increase the positive rate of blood culture.

Key word Blood culture Aerobic bottles Anaerobic bottles Evaluation studies

Blood infection, such as bacteremia and sepsis are the common systemic disease in clinics, which will threaten patient's life if not get therapy in time. Therefore blood culture is the "Golden standard" on blood infection in clinic diagnosis. Bact/ALERT® 3D Fully Automated Blood Culture Analyzer, FA blood culture bottle and FN blood culture bottle are the widely used blood culture equipment in clinic laboratories. Considering imported analyzer and its consumables are very expensive, which is very difficult to popularize in medium and small hospitals, we compare the function and detection capacity of a local brand blood culture system and imported blood culture system with same patient so as to supply reference to clinic application.

Material and method

Material

Specimen source and collection: samples are collected from patients in need for blood culture test from department of Infection, Gastroenterology, Surgery ICU, Dermatology in Shanghai Ruijin Hospital from march to August 2012. Nurse is required to collect blood correctly: sterilize their hands and patient's skin before collection; collect specimen when patient in clinical symptoms (such as high fever, first stage of shiver); collect it before patients taking antibiotics, or collect before patients taking antibiotics next time if antibiotics has already been taken previously; Every patient should be collected with two bottles and two sides. That is: one aerobic bottle and one anaerobic bottle from DL Medical *Bt-64* (FA/FN); While one aerobic bottle and one anaerobic bottle from BioMerieux *Bact/Alert® 3D* (FA/FN); Collect sample volume with 5ml/bottle for adult and send them to laboratory for testing within 2 hours.

1. Equipment:

Bact/Alert® 3D Fully Automated Blood Culture Analyzer produced by Biomerieux.

Bt-64 fully Automated Blood Culture Analyzer produced by Zhuhai DL Medical

2. Blood culture bottle:

Bact/Alert® 3D FA aerobic bottle and /FN anaerobic bottle produced by BioMerieux.

Bt-64 FA aerobic bottle and FN anaerobic bottle produced by Zhuhai DL Medical.

Method

1. Collect the blood of same patient at two sides at same time, then dispense the blood into two sets of aerobic and anaerobic bottles (dispense 5ml blood collected from left side in both *Bact/Alert® 3D* and *Bt-64* aerobic bottle respectively; dispense 5ml blood collected from right side

in both *BacT/Alert*[®] 3D and *Bt-64* anaerobic bottle respectively.

2. Place blood culture bottle in *BacT/Alert*[®] 3D and *Bt-64* blood culture analyzer abide by operating regulations for incubation and measurement.
3. Set culturing time as 5 days. Take out positive bottle immediately when it gave warns of positive, record positive report time, then perform Gram stain and transferring culturing. Aerobic bottle are cultured in blood agar and general chocolate agar incubates 18-24 hours at 35°C and 5%CO₂; Anaerobic bottle are cultured in Anaerobic blood agar, incubates 48 hours at anaerobic environment, and perform oxygen tolerance test in aerobic atmosphere at the same time. Deliver last report after isolation and identification of bacteria. If machine shows positive but smear and culture result is negative, then final judge as negative (false positive). After 5 days culturing, if it reports negative, take out blood culture bottle and record it is negative.
4. All positive isolated strain is identified by VITEK 2, make drug sensitivity test with K-B method, at last issue the final report.
5. Statistical analysis: establish EXCEL for all data; analyze result with SPSS statistical software.

Result

1. Overview, excluding the patients that are not in line with above standards, or polluted bottles, totally 360 sets blood culture bottles are selected (180 sets of *Bt-64* and *BacT/Alert*[®] 3D bottles respectively, among which, 180 bottles of aerobic and anaerobic bottles are applied, respectively) from Department of Infection, Gastroenterology, Surgery ICU, Dermatology in Shanghai Ruijin Hospital from march to August 2012. There are 31 sets of blood culture bottle showed positive, with Gram stain and culture to detect bacteria, total positive rate is 8.6% and detected 32 strains of bacteria, in which 15 sets are from *Bt-64* blood culture bottle, with positive rate 8.3%; 16 sets are from *BacT/Alert*[®] 3D blood culture bottle, with positive rate is 8.9%. Furthermore, 6 sets (5 sets of *Bt-64* blood culture bottle and 1 set of *BacT/Alert*[®] 3D blood culture bottle) reported positive, but after 2 days culturing there is no bacteria growth, which means false positive. Meantime, total 323 sets of blood culture bottle reported negative, negative rate is 89.7%. 160 sets of *Bt-64* blood culture bottle reported negative, negative rate is 88.9%; 163 sets of *BacT/Alert*[®] 3D blood culture bottle reported negative, negative rate is 90.6%. Statically, $X^2=0.015$, $P>0.05$, both positive and negative detection rate of two kinds of blood culture bottle show no significant difference. See

Table 1

Table 1. Overview on positive/negative results of *Bt-64* and *BacT/Alert*[®] 3D blood culture bottle

Group	Result		Total
	Positive case	Negative case	
<i>Bt-64</i> bottle	15(8.6%)	160(91.4)	175
<i>BacT/Alert</i> [®] 3D bottle	16(8.9%)	163(91.1)	179
Total	31	323	354

2. Comparison of overall culture result from *Bt-64* and *BacT/Alert*[®] 3D blood culture bottle: take *BacT/Alert*[®] 3D blood culture bottle as Golden standard, *Bt-64* is evaluated by sensitivity, specificity, false positive rate and false negative rate (see Table 2).

Table 2. Comparison of results between *Bt-64* and *BacT/Alert*[®] 3D

<i>Bt-64</i> bottle	<i>BacT/Alert</i> [®] 3D bottle			Sensitivity	Specificity	False positive	False negative	Association
	Positive case	Negative case	Total					
Positive case	14	5	19					
Negative case	2	157	159	87.5%	96.9%	3.1%	12.5%	96.1%
Total	16	162	178					

3. Comparison of bacteria-detection average growth time between *Bt-64* and *BacT/Alert*[®] 3D

From table 2, there are 14 sets reported positive for both *Bt-64* and *BacT/Alert*[®] 3D in common. It means, for the same patient's specimen, both *Bt-64* and *BacT/Alert*[®] 3D successfully detect bacteria. In such 14 sets positive specimen, for each one, if both aerobic and anaerobic report positive, earlier one is chosen as right one. By comparing the average growth time (Table 3), and applying in paired t-test, the Sig=0.929>0.05, t=0.091, it indicates there is no significant difference between these two bottles.

Table 3. Comparison of bacteria-detection average growth time between *Bt-64* and *BacT/Alert*[®] 3D

Bacteria Name	Average Growth Time (H)	Difference(H)
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	<i>Bt-64</i>	<i>BacT/Alert</i> ® 3D	
Hemolytic Staphylococcus	28.6	20.2	8.4
Escherichia coli	4.1	4.1	0.0
Enterococcus faecalis	15.1	14.4	0.7
Staphylococcus aureus	6.5	8.9	-2.4
Listeria monocytogenes	27.6	22.1	5.5
Staphylococcus epidermidis	17.5	17.5	0.0
Staphylococcus lentus	28.6	59.3	-30.7
Klebsiella oxytoca	1.9	2.2	-0.2
Escherichia coli	19.0	1.9	17.0
Klebsiella pneumoniae	12.2	12.2	0.0
Klebsiella pneumoniae	11.3	10.8	0.5
Acinetobacter baumannii	12.5	14.2	-1.7
Acinetobacter baumannii	19.4	13.0	6.5
Staphylococcus aureus	9.4	9.4	0.0

4. Comparison of Aerobic/Anaerobic bottle (FA/FN) between BioMerieux *BacT/Alert*® 3D and DL Medical *Bt-64*

Each patient's blood samples are divided into two parts and injected into FA/FN bottles of *BacT/Alert*® 3D and *Bt-64* individually. Comparison of association or consistency is obtained. Positive rates of Aerobic bottle for *Bt-64* and *BacT/Alert*® 3D are 5% and 7.8%, respectively; Negative rates of Aerobic bottle *Bt-64* and *BacT/Alert*® 3D are 95% and 92.2%, respectively. Meanwhile, positive rates of Anaerobic bottle for *Bt-64* and *BacT/Alert*® 3D are 10% and 7.2%, respectively; Negative rates of Anaerobic bottle *Bt-64* and *BacT/Alert*® 3D are 90% and 92.8%, respectively. By SPSS statistics software, Kappa association is applied for two groups of data. Rules-of-thumb: if Kappa value is equal or more than 0.75, it indicates high association. According to results, Aerobic and Anaerobic groups show Kappa value 0.6759 and 0.7535, respectively. Anaerobic group indicates excellent association (0.7535). And comparatively, Aerobic group (0.6759) is a little bit lower but still substantial. (Table 4)

Table 4. Consistency/Association comparison of Aerobic/Anaerobic bottle (FA/FN) between BioMerieux *BacT/Alert*® 3D and DL Medical *Bt-64*

	DL Medical <i>Bt-64</i>	BioMerieux <i>BacT/Alert</i> ® 3D		Total
		Positive Results	Negative Results	
Aerobic bottle	Positive Results	8	1	9

	Negative Results	6	165	171
	Total	14	166	180
Anaerobic bottle	Positive Results	12	6	18
	Negative Results	1	161	162
	Total	13	167	180

Septicemia is one of the main causes of death for patients with severe infection. According to statistics worldwide, there are hundreds of thousands of Sepsis patients. Take the USA as example, the Sepsis number increases by 139% during past ten years. In China, positive rate in blood culture is around 20%. The reasons for low detection rate are complicated: such as disinfection job before blood sampling; whether patient's symptom matches blood test standard; whether time for sampling is correct; sampling place, sampling volume and number of bottles for blood culture; whether technician follows up protocol etc. In China, the main systems for blood culture are from BD (*BACTEC 9000*) and BioMerieux (*BacT/Alert® 3D*). The instrument and reagents are imported and price is expensive. Only big general hospitals and developed region can afford it. Rest other middle or small hospitals cannot sustain such high cost. Therefore, DL Medical's *Bt* series blood culture system, which are developed by local manufacturer with own technology and patents, are highly respected. The system has already passed clinical application and tests with good results.

In this trial, totally 360 sets of blood culture bottles (720 bottles) are involved. 180 sets (360 bottles) are from DL Medical *Bt-64*. 180 sets (360 bottles) are from BioMerieux *BacT/Alert® 3D*. 31 sets reported positive, with confirmation by staining and transferring afterwards. Total positive rate is 8.6%. Out of 31 positive, 15 sets are from DL Medical *Bt-64*, 16 sets are from BioMerieux *BacT/Alert® 3D*. Positive rate for each is 8.3% and 8.9%, respectively. Correspondingly, the negative rate for each system is 88.9% and 90.6%, respectively. The total positive and negative detection rates are very similar to each other. Suppose taking BioMerieux *BacT/Alert® 3D* as golden standard, DL Medical *Bt-64* correlation is up to 96.9%, with false positive 3.1%. In the same time, the sensitivity of DL Medical *Bt-64* is 87.5%, relatively lower, while the false negative is 12.5%, relatively higher. It might be related with limited sample volume. More tests are required for further validation. In terms of reporting time, both systems demonstrate similar average growth time. If we compare detection rate, for Anaerobic bottle, DL Medical *Bt-64* is highly correlated with BioMerieux *BacT/Alert® 3D*. For Aerobic bottle, DL Medical *Bt-64* is also good.

Some previous papers indicated that there is reporting delay or undetected case towards *Candida* etc bacteria in BioMerieux *BacT/Alert® 3D* system. In this trial, even though only 32 positive strains are separated, DL Medical *Bt-64* reported a case of yeast, while BioMerieux *BacT/Alert® 3D* showed negative results.

Contamination in blood culture is a long time problem. Weinstein found that, almost half (42.9%) reports are considered contamination. In this experiment, we kicked out all samples polluted in order to guarantee the reliability of results.

In conclusion, accuracy of blood culture mainly relies on quality of blood culture bottle. High quality product is the assurance of test results. In this report, we validated and compared data for both BioMerieux *BacT/Alert*® 3D and DL Medical *Bt-64* system. In statistics, there is no obvious discrepancy for positive detection rate and negative detection rate. In addition, there is no obvious discrepancy for reporting time. Comparing with BioMerieux *BacT/Alert*® 3D, DL Medical *Bt-64* system demonstrates high specificity and correlation >96%. However, sensitivity and undetected rate are expected to improve furthermore. Finally, in terms of consistency, Anaerobic bottle is better than Aerobic bottle

Reference

1. Lynn LJ, Duane RH, Clinton KM, et al. Detection of Simulated Candidemia by the BACTEC 9240 System with Plus Aerobic/F and Anaerobic /F Blood Culture Bottles [J]. J Clin Microbiol, 2003, 10 (41): 4714-4717
2. JF Yang, JH Li, X Wang et al. Research on isolates distribution and drug resistance from 6445 blood samples [J]. China Hospital Infection Magazine, 2003, 13(6): 575-577
3. YC Pu, HW Lu, H Li, et al. Analysis of 730 specimen in Aerobic bottles of Automated Blood Culture System [J]. Shanghai Medical Diagnostic Magazine, 2000, 15(3): 168-169, 153.
4. Klaerner, HG, Eschenbach U, Kamereck K, et al. Failure of an automated blood culture system to detect nonfermentative gram-negative bacteria. J Clin microbial, 2000, 38: 1036-1041.
5. Smith JA, Bryce EA, Ngui-Yen JH, et al. Comparison of BACTEC 9240 and BacT/Alert blood culture systems in adult hospital. J Clin Microbiol, 1995, 33: 1905-1908.
6. Arlet G, Garido E, Lagrange P. Multicenter Clinical Evaluation of the BACTEC NR Using Resin Bottl. And VITAL Blood culture systems, ASM, Las Vegas, 1994. 8-9.
7. Y Cai, Contamination and Judgment of Blood Culture [J]. Medicine Information, 2009, 1(10): 221-222.
8. SZ Nong, SF Chen, LH Wei, et al. Analysis and Comments on Septicemia of Inpatient by two kinds of Aerobic blood culture system [J]. Guangxi Medicine, 2001, 23(6): 1368-1369.