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Comparison of Different Media for the Quantitative Detection of Staphylococcus Aureus

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Abstract: In accordance with the steps of the second method of GB 4789.10-2016, culture experiments were carried out on standard strains of Staphylococcus aureus, isolates from food samples using S. aureus chromogenic medium and Baird-Parker medium. There was no difference between the quantitative detection results of the two media in the concentration of S. aureus from 20 to 200 CFU/mL (P>0.05), the colonies on the Baird-Parker plate were smaller, black and without turbid band when cultured for 24 h. The characteristics of typical colonies were not obvious, and typical colonies appeared when the culture was prolonged to 48 h. The colonies appeared with typical characteristics of growth, black and turbid band; the colonies showed pinkish-purple when cultured for 18 h on the colour-developing plate, and the detection efficiency of colour-developing plate was superior to that of Baird-Parker plate. When cultured for 18 h on the chromogenic plate, the colonies were pinkish-purple, and the detection efficiency of the chromogenic plate was better than that of the Baird-Parker plate, and the incubation time could be shortened by about 24h. The quantitative detection effect of the chromogenic medium and the Baird-Parker medium on Staphylococcus aureus was comparable, but the chromogenic medium could shorten the cultivation time and had a higher efficiency, and the sensitivity and specificity of chromogenic medium for the detection of the strains preserved in the laboratory were higher than that of the Baird-Parker medium. The sensitivity and specificity of the colour-developing medium were higher than those of the Baird-Parker medium.

1. Introduction

Staphylococcus aureus belongs to the genus Staphylococcus and is commonly found in air, food, sewage and other environments[1], and has a strong ability to adapt to different environments, surviving in liquid media containing 150g-L^{-1} sodium chloride and growing unaffected in TSB agar with a mass concentration of 130g-L^{-1} sodium chloride. It poses a potential threat to human and animal life and health, food safety and public health, and can also cause a variety of human infections. It is a common clinical pathogen associated with community- and hospital-acquired diseases, and mainly causes a variety of diseases such as skin and soft-tissue infections, endocarditis, osteomyelitis, bacteraemia, and lethal pneumonia[2] . Staphylococcus aureus invades the organism and produces a variety of toxins and virulence factors that evade the body's immune system,

causing local and systemic infiltrative infections[3] . Staphylococcus aureus can usually colonise the anterior nostrils asymptomatically in 30-50% of the population, and nasal carriers of Staphylococcus aureus have a risk of infection that is 2-12 times higher than that of non-carriers[4] . Staphylococcus aureus and its enterotoxins are one of the most common causes of bacterial food poisoning, and there are hidden dangers in animal breeding, food production and processing, and food regulation. Enterotoxins are a group of extracellular egg A plasmids composed of structurally related, virulence-similar, antigenically different, and thermally stable plasmids, and they are still immunologically and biologically active even when boiled at 100 ℃ for 30 min, and are the main factors leading to S. aureus food poisoning. Staphylococcus aureus is the main factor leading to food poisoning, and food poisoning caused by it has become a worldwide public health problem, so it is very important to monitor S. aureus in food. Rapid and accurate detection of S. aureus is a key factor in ensuring food safety and protecting humans from foodborne diseases. Traditional culture-based assays are the gold standard for the detection of foodborne pathogens, but are time-consuming and labour-intensive. Therefore, the selection of isolation medium with good specificity and high specificity is the first critical step in the isolation and identification of S. aureus. In recent years, many studies have shown that the qualitative isolation of Staphylococcus aureus by chromogenic medium has better clinical results, but fewer reports have been made on its quantitative detection effect. Therefore, this study compares the quantitative detection effects of S. aureus chromogenic medium and Baird-Parker medium on S. aureus, with a view to providing technical support for food safety risk assessment, which is of great significance for ensuring food safety and reducing foodborne diseases.

2. Materials and methods

2.1 Main media, reagents and instruments

The materials used in the study can be seen in Table 1.

Table 1: Main culture media, reagents and instruments

makings	factory owners
7.5 per cent sodium chloride broth	
Baird-Parker Agar Plates	
Blood agar plate	
Colony Counting Agar Plates	
Tryptone Soya Agar Plate	
Brain and Heart Leachate Broth	Beijing Landbridge Technology Co.
Staphylococcus aureus chromogenic medium	CHROMagar, Comagar, France
Lyophilised blood racks	Guangdong Hankai Microbial Technology Co.
Gram-positive bacteria identification slat	BD USA, Inc.
BAGMIXER 400 Homogeniser	Interscience, USA
750 thermostatic incubator	Memmert, Germany
1378 Class II biological safety cabinet	
902 Ultra Low Temperature Refrigerator	Thermo Fisher, USA
Phoenix M 50 Fully Automated Microbiological Identification System	BD USA, Inc.
CX33 Biological Microscope	Olympus Ltd.
1KA Vortex genius 3 vortex oscillator	De MIKA
Micro Stainer Automatic Gram Stainer	Shanghai Haoxin Biotechnology Co.

2.2 Test strains

Standard strains: Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Staphylococcus epidermidis, Enterococcus faecalis, Salmonella typhimurium. The above strains are stored in magnetic bead storage tubes at -70 ℃.

Food sample isolates: 30 strains of Staphylococcus aureus, 5 strains of Staphylococcus capitatus, 3 strains of Staphylococcus vivax, 10 strains of Staphylococcus kochneri subspecies, 7 strains of Staphylococcus xylosus, 5 strains of Staphylococcus spp, 5 strains of Staphylococcus bartholomeus, all of them are isolated from food samples of the Laboratory, and the strains above were stored in magnetic bead storage tubes at -70 ℃.

2.3 Methodology

The standard strain of Staphylococcus aureus and 30 food samples were isolated from Staphylococcus aureus and the bacterial suspension was prepared with reference to GB 4789.28-2013 Quality Requirements for Culture Media and Reagents for Microbiological Examination of Foods, the magnetic beads were inoculated into the TSA plate, incubated at (36±1)℃ for 18-24 h for resuscitation, and then a single colony was inoculated into the BHI broth culture medium. And (36 ± 1) ℃ culture 18 ~ 24 h, with a concentration of 0.85% of sterile saline continuous gradient dilution of the cultured BHI broth, control the bacterial concentration of 20 ~ 200 CFU / mL, with reference to GB 4789.10-2016 Staphylococcus aureus plate counting method, aseptic aspiration of 1mL of diluted bacterial suspension to 0.3, 0.3, 0.4 mL of inoculation volume Add 3 pieces of Baird-Parker agar plates respectively, spread the plates with a sterile spreading stick, after spreading, the plates were left to stand for 10 min to be absorbed and then turned over and inverted, and then incubated at (36±1)℃ for 24-48 h to determine the number of S. aureus. The number of Staphylococcus aureus in the suspension was determined by the same method using Staphylococcus aureus chromogenic plate.

2.4 Statistical methods

The obtained data were collated into tables and uploaded to the database, and the data were processed by professional staff using SPSS version 26.0 statistical software. The obtained data were statistically processed, and the measured data obeying normal distribution were expressed as (mean ± standard deviation) and compared by t-test, and $P<0.05$ was considered statistically significant.

3. Results

3.1 Comparison of the quantitative results of the two culture media for S. aureus

There was no difference between the quantitative detection results of the two media in the concentration of S. aureus from 20 to 200 CFU/mL ($P>0.05$), see Table 2.The colonies on the Baird-Parker plate were small, black, without turbid band when cultured for 24 h, and the typical growth characteristics of colonies were not obvious, and the colonies appeared the typical growth characteristics of colonies when cultured for 48 h. The colonies appeared pinkish purple when cultured for 18 h on the chromogenic plate, and it can be seen that the detection efficiency of chromogenic plate was better than that of Baird-Parker plate for about 24 h. See Figure 1. When cultured for 48 h, the colonies showed typical growth characteristics with black colour and turbid bands; when cultured for 18 h on the chromogenic plate, the colonies were pinkish-purple, which showed that the detection efficiency of the chromogenic plate was better than that of the

Baird-Parker plate, and the incubation time could be shortened by about 24 h. See Fig. 1.

Table 2: Comparison of quantitative test results for Staphylococcus aureus ($\bar{x} \pm s$, CFU/mL)

Staphylococcus aureus concentration	Number of colonies on colour-developing plates	Baird-Parker plate colony counts	*t*	*P*
20-100CFU/mL	58.46±2.67	61.43±2.05	1.973	0.084
100-200CFU/mL	116.72±4.31	122.17±4.01	2.070	0.072

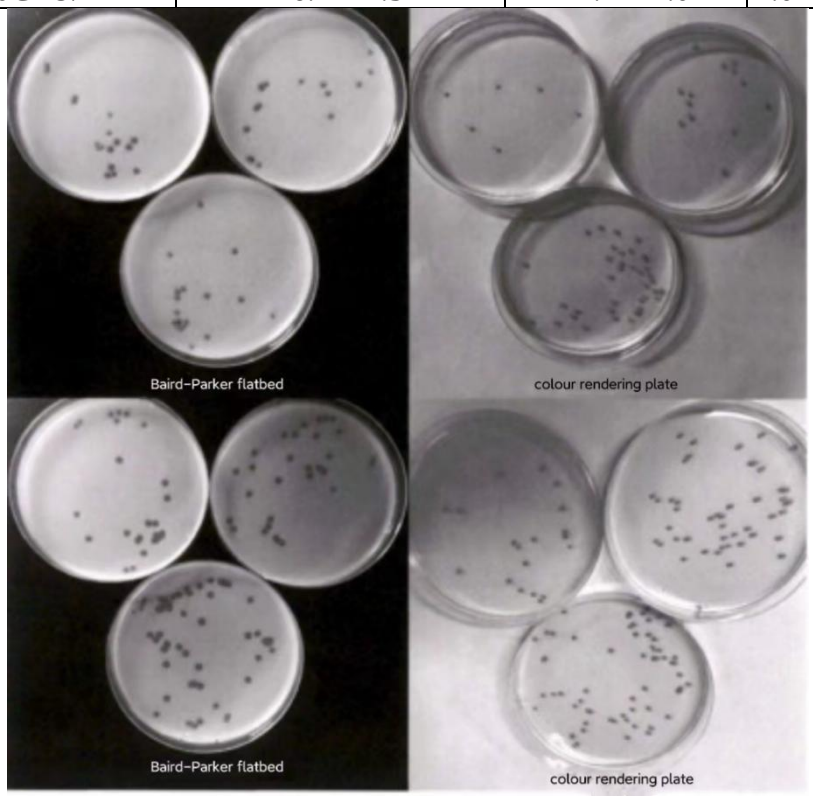

Figure 1: Enumeration results of Staphylococcus aureus in two media

3.2 Comparison of the results of laboratory preserved strains tested for growth in medium

The chromogenic medium was more sensitive and specific than the Baird-Parker medium for the detection of laboratory preserved strains. See Table 3.

Table 3: Effectiveness of the two culture media on the detection of laboratory preserved strains

culture medium	Staphylococcus aureus growth (strain)	Sensitivity (per cent)	Specificity (per cent)
colour rendering plate	30	96.7	96.5
Baird-Parker flatbed	30	90.2	91.0

4. Discussion

Staphylococcus aureus is a Gram-positive rod-shaped bacterium that is widely found in nature and is considered one of the most common foodborne pathogens[5-6] . With the general increase in antibiotic resistance of S. aureus, over the years, S. aureus has become a leading cause of complex

infectious diseases that face serious threats in hospitals and communities[7-8] . S. aureus can cause bacterial infections in humans and animals, and due to its ability to produce coagulase, it can produce localised septic infectious diseases, including mild skin and soft tissue infections such as respiratory, peritoneal enteritis, and endocarditis; as well as bloodstream infections, sepsis, toxic shock syndrome (TSS), and other systemic infections, which have consequences that seriously jeopardise human health[9] . Under the right conditions, S. aureus can produce enterotoxins that are destructive to the intestinal tract and cause food poisoning, mainly from contaminated food such as milk, meat, eggs and leftovers[10] . The following conditions have been reported to be required for the production of S. aureus enterotoxin: first, the presence of S. aureus that carries an enterotoxin-positive genotype; second, the presence of physicochemical properties in the food composition that are favourable to the growth and production of S. aureus; and third, the presence of suitable temperatures and sufficient time to allow the bacteria to grow to more than 105 CFU/g to produce the toxin. Enterotoxins are resistant to high temperature, pH, and a variety of proteases, and cannot be completely inactivated even after food processing[11] , therefore, controlling the production of enterotoxins is the key to preventing S. aureus food poisoning outbreaks. Dairy and meat products in food are the most common sources of S. aureus contamination[12] , in which the distribution of S. aureus enterotoxin genotypes on the source of contamination has been studied[13] , but most of them have only carried out qualitative testing, and the specific expression of different enterotoxin genotypes in food matrix has not been clarified. China released the National Standard for Food Safety Pathogenic Bacteria Limit in Prepackaged Food GB 29921-2021 and set the national standard for the limit of S. aureus. The maximum safety limit value for acceptable level of S. aureus contamination should not exceed 100 CFU/g in all types of food product categories, therefore, rapid detection and identification of S. aureus is the key to ensure food safety.

At present, the selective isolation of S. aureus mostly uses Baird-Parker medium, due to the sensitivity and specificity of Baird-Parker medium is not ideal, some other staphylococcus spp. in food are also easy to grow, and the morphology is similar to that of S. aureus, so it is difficult to differentiate the suspected strains directly from Baird-Parker medium. It is difficult to distinguish the suspected strains directly from Baird-Parker medium, and the subsequent confirmation and identification according to GB 4789.10-2016 also need to pick the suspected target strains for purification, Gram staining microscopic examination and plasma coagulase test, which will take about another 3d to identify[14-15] , which not only increases the workload of the inspectors, but also reduces the efficiency of the test. Localized chromogenic media is a biochemical reaction-based microbial identification technique, the principle is based on the differences in intracellular enzyme reaction conditions of different microorganisms as a basis for taxonomic identification,specific substrates are added to the isolation medium, which consist of substances metabolizable by the microorganisms as well as chromogenic groups, in the presence of microbe-specific enzymes, the chromogenic groups free and develop color, by observing the colour of the colony, it is possible to identify the strain[16] . Moreover, the chromogenic plate does not require high requirements for detectors, and can be detected by simply observing the colony morphology and colour, which is more efficient. The results of this study show that the quantitative detection of S. aureus by the two media is comparable, but the colour-developing medium is more efficient and can effectively shorten the cultivation time.

5. Conclusion

Both media have good quantitative detection of S. aureus, but the incubation time of the chromogenic media is shorter, and the sensitivity and specificity of the detection of laboratory-preserved strains are higher, which makes it more valuable for clinical application.

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