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The effect of broth media on pneumococcal growth and the latex serotyping result

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Abstract

The aim was to test Todd–Hewitt broths (TH-broths) for the ability to propagate pneumococci and thereafter to evaluate the serotyping result obtained by the Pneumotest-Latex kit (SSI).

TH-broths from four different producers (Oxoid, Sigma, Difco, and SSI) were tested and compared separately and with Serum broth (SSI). Twenty-three pneumococcal strains (different serotypes) were inoculated into the broths with start inoculums of 10^1, 10^3, and 10^6 CFU/ml. After incubation, overnight viable bacterial counts and visible growth were recorded. All pneumococci were serotyped with the Pneumotest-Latex kit.

After incubation, the bacterial counts in all TH-broths were within the range of log 4.65–log 7.76 CFU/ml, while Serum broth showed an average growth ranging from log 8.05–log 8.90 CFU/ml. Comparing the growth of the four TH-broths showed no significant differences. In general, Serum broth had a more pronounced visual growth than each of the four TH-broths. Serotyping with Serum broth showed in general positive and correct latex typing results for all serotypes and initial inoculum, while the outcome of the TH-broths showed some false negative results depending on inoculum and serotype.

Overall the Serum broth was found to be superior to the four TH-broths tested both with regard to CFU/ml and when used with the Pneumotest-Latex kit. However, if the Pneumotest-latex kit is only used on broths with visible growth as stated in the instruction manual, then the differences between the performances of the broths from the different producers was not significant and all broths could be used for Pneumotest-Latex typing.

Keywords: Serum broth; Todd-Hewitt broth; Latex assay; Serotyping; Pneumococci; Neufeld

1. Introduction

The bacterium *Streptococcus pneumoniae* (pneumococcus) is a major cause of invasive diseases and

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upper respiratory tract infections (Arai et al., 2001). Prevention strategies against pneumococcal infections have been based on the immunogenicity of the polysaccharide capsule. At present, 90 different capsular types have been described and type-specific polysaccharide antibodies for typing have been raised in rabbits (Henrichsen, 1995).

The "gold standard" method for serotyping of pneumococci is the capsular reaction test also known as the Quellung reaction (Neufeld test; Sorensen, 1993, Henrichsen, 1995) that uses blood agar plates as growth medium before microscopy of the pneumococci (Sorensen, 1993). However, the need for easier typing methods has presented new methods which use broths instead of blood plates for culture of pneumococci (Slotved et al., 2004).

During the development of the pneumococci serotyping kit Pneumotest-Latex (Statens Serum institute (SSI), Copenhagen, Denmark) TH-broth manufactured at SSI was used (SSI Diagnostica, Hillerød, Denmark). It was, however, found not to be as efficient as the SSI standard Serum broth (SSI Diagnostica) for growth of pneumococci (Slotved et al., 2004). Since a study has shown that TH-broths offer a high concentration of free antigens (Lafong and Crothers, 1988), they have been commonly used as a standard medium in many laboratories around the world for growth before routine serotyping (Marshall et al., 1993, Lafong and Crothers, 1988).

The aim of this study was to test four different TH-broths and Serum broth for their ability to propagate pneumococci after overnight incubation (18 and 24 h) and thereafter to evaluate the latex serotyping result obtained by the Pneumotest-Latex kit.

2. Materials and methods

2.1. Strains and media

All strains were obtained from the Statens Serum Institut *Streptococcus* strain collection (WHO Collaborating Centre for Reference and Research on Pneumococci, the Streptococcus Unit, SSI, Copenhagen, Denmark).

Twenty-three pneumococcal reference strains representing the 23 serotypes included in the polyvalent vaccine were used for the test of the five broths.

All strains were stored either lyophilized or in nutrient beef broth with 10% glycerine (SSI Diagnostica) at −80 °C and 10% blood agar plates (horse blood; SSI Diagnostica) were used for subculturing. Plates were incubated overnight at 33°C–37°C in CO_2 and stored at 5 °C for a week before being discharged.

Broth media: 3 ml TH-broth from four different producers (Oxoid CM189, Sigma T-438, Difco, and SSI Diagnostica) and 3 ml Serum broth (SSI Diagnostica) were used.

2.2. CFU counts

From the overnight plate culture, pneumococci were suspended in physiological saline in a concentration of 1–2×10^8 CFU/ml (OD_{540}=0.28) measured by a colorimeter (Sherwood, nb. 254). From the bacterial suspension a 10-fold serial dilution from 10^7 to 10^3 CFU/ml was prepared in physiological saline, and 30 μl of the specific dilution was inoculated into the five different broths (preheated to 33°C–37°C) resulting in start inoculum of approximately 10^1, 10^3, and 10^6 CFU/ml. Broths were all incubated overnight at 33°C–37°C without the use of CO_2. The cell counts were performed after 18 and 24 h of incubation by withdrawing a 100 μl broth sample and 10-fold diluting it in physiological saline. 20 μl of each dilution were added to a 10% blood agar plate, and colonies were counted after incubation overnight at 33°C–37°C with CO_2.

2.3. Performance of the latex agglutination test

The latex agglutination was performed as described by Slotved et al. (2004). Briefly, an aliquot of 10 μl of an overnight pneumococcal broth culture was mixed with 10 μl latex reagent on a reagent card (SSI), and thereafter the card was manually rocked for 5–10 s. A positive reaction could be read with the naked eye within 5–10 s. Samples from all broths and inoculums were tested for their positive reaction in the corresponding latex pools using Pneumotest-Latex (SSI). Interpretation of the serotype/serogroup was done by use of the chessboard system (Pneumotest-Latex).