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Video Article

Capsular Serotyping of *Streptococcus pneumoniae* Using the Quellung Reaction

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Abstract

There are over 90 different capsular serotypes of *Streptococcus pneumoniae* (the pneumococcus). As well as being a tool for understanding pneumococcal epidemiology, capsular serotyping can provide useful information for vaccine efficacy and impact studies. The Quellung reaction is the gold standard method for pneumococcal capsular serotyping. The method involves testing a pneumococcal cell suspension with pooled and specific antisera directed against the capsular polysaccharide. The antigen-antibody reactions are observed microscopically. The protocol has three main steps: 1) preparation of a bacterial cell suspension, 2) mixing of cells and antisera on a glass slide, and 3) reading the Quellung reaction using a microscope. The Quellung reaction is reasonably simple to perform and can be applied wherever a suitable microscope and antisera are available.

Video Link

The video component of this article can be found at <http://www.jove.com/video/51208/>

Introduction

Streptococcus pneumoniae (the pneumococcus) is responsible for the death of an estimated 800,000 children under the age of five annually, with the burden of disease falling predominantly in resource-poor countries¹. Pneumococcal diseases range in severity from localized infections such as acute otitis media to severe life threatening infections such as sepsis, meningitis and pneumonia². Over 90 capsular serotypes of pneumococci have been described^{3,4}. Current pneumococcal vaccines provide protection against the serotypes that are responsible for the majority of invasive disease^{5,6}. However, widespread use of vaccines has been associated with replacement of serotypes included in the vaccine by nonvaccine serotypes, both in nasopharyngeal carriage and invasive disease^{7,8}. This highlights the importance of serotyping for epidemiological surveillance and long-term vaccine impact studies⁹.

The gold standard method for pneumococcal typing is the capsular reaction test, known as the Quellung reaction, first described by Neufeld in 1902 (see Austrian¹⁰). A high degree of concordance has been found between laboratories, and between the Quellung reaction and other serotyping methods¹¹⁻¹³. There are several variations of the Quellung reaction^{10,14-16}. The method described here does not require the use of a counter stain or the oil immersion lens; rather, a drop of prepared specimen is mixed with the typing serum and examined immediately under 400X magnification.

The Quellung reaction is usually performed using commercially available antisera. The method described here uses less antisera compared with some other methods^{16,17}, making it more cost effective. Once an isolate is identified as a pneumococcus, it is sequentially tested with antisera pools until a positive reaction is observed. Each pool antiserum contains different mixtures of antisera raised against 91 pneumococcal serotypes¹⁸. Once the pool is established, the individual type and group antisera that are included in the reactive pool are tested individually in sequence. Type antisera generally react with a single serotype (e.g. Type 1 antiserum reacts with serotype 1), whereas group antisera react with all the serotypes in a particular group (e.g. Group 22 antiserum reacts with serotypes 22F and 22A). Some serotypes within groups are further differentiated using factor antisera (e.g. Serotype 22F reacts with factor 22b, but not 22c). In this case testing is continued with all relevant factor antisera until serotype is determined^{19,20}. The Quellung reaction is simple and quick¹⁴ and can be performed in any laboratory that is equipped with a good quality microscope and appropriate set of antisera.

Protocol

1. Preparation of Cell Suspension for Typing

- Using a sterile disposable 1 μ l loop, take a small sweep of fresh overnight pure culture of pneumococci grown on solid nonselective horse blood agar and inoculate 100 μ l Heart Infusion (HI) broth. The suspension should appear to be just visibly turbid. Gently agitate the tube to emulsify.
Note: Other media such as Brain Heart Infusion broth may also be suitable. Addition of serum (e.g. a final concentration of 10% (v/v) horse serum) and incubation at 37 °C for approximately 1 hr before use may be used to enhance the reaction.

2. Checking Density of Cell Suspension

The cell suspension prepared in step 1.1 will also be used as the negative control, and should be checked for cell density before adding the typing sera.

- Using a 1 μ l loop, transfer a drop of the inoculum prepared in step 1.1 onto a glass microscope slide.
- Using a pair of forceps, place a small circular coverslip on the suspension.
- Using phase-contrast setting, observe the preparation microscopically under 400X magnification, to check the density of the suspension (**Figure 1A**).
 - If the inoculum is too heavy (**Figure 2A**), add more broth to the cell suspension made in step 1.1 to dilute it. Gently mix again.
 - If the inoculum is too light, for example, there are <50 cells visible in the microscopic field (**Figure 2B**), add more bacteria to the cell suspension preparation made in step 1.1 and gently mix.
Note: With experience, the density of the cell suspension can be judged by shaking and holding up to the light, where it should appear to be just visibly turbid.
- Repeat steps 2.1-2.3 until a suitable number of cells are visible in the microscopic field.
Note: Glass slides and coverslips present a sharps hazard. After use, slides and coverslips are contaminated with infectious material and must be disposed into a biohazard sharps container.

3. Typing

- Transfer ~1 μ l of the prepared inoculum (e.g. using a 1 μ l loop) onto the glass microscope slide. Discard the loop into the biohazard sharps container.
- Add an equal volume (1 μ l loopful) of room-temperature antiserum onto the slide and mix both drops well with the loop.
- Using a pair of forceps, place a small circular coverslip on the suspension, taking care not to touch the drop. Place the coverslip on the suspension immediately after the addition of antisera to prevent the fluid on the slide from drying out.
- Using a microscope, observe the suspension under phase-contrast with 400X magnification. A negative Quellung reaction is observed when little or no 'swelling' of the pneumococcal cells can be seen at 400X magnification, similar to what is observed in the pneumococcal cell suspension with no antisera added (negative control). A positive Quellung reaction is observed when the cells appear enlarged or 'swollen' and more visible when compared to the cells in the negative control.
Note: Equivocal reactions should be investigated further, for example by examining the reaction after 5-10 min incubation at room temperature, or by repeating the reaction with more antiserum.
- Conduct pneumococcal serotyping with successive testing of individual antisera pools until a positive reaction is observed. Alternatively, pools can be applied in 'rounds' depending upon local incidence of serotypes.
Note: Testing in 'rounds' ensures that the pools containing antibodies to the most common serotypes are tested first, minimizing effort and cost. Concurrent testing of several pools also provides additional negative controls, which can be useful in interpretation. If all the pools in a particular 'round' are negative, test the isolate with subsequent rounds of pools.
- Test the isolate using the appropriate type or group antiserum contained within the reactive pool. Where appropriate, use factor antisera to further differentiate the serotype.
Note: Keys to pneumococcal antisera supplied by the manufacturer are used in the selection of appropriate pool, type, group or factor antisera and interpretation of results (including any cross-reactions).
- If negative results are obtained with all pools, test the isolate with Omniserum reagent (contains antibodies against 91 serotypes²¹). If a positive Quellung reaction is still not observed when testing a pneumococcal isolate with the Omniserum reagent, this may be because the isolate is expressing little or no capsule, or that antibodies against the serotype are not represented in the Omniserum. The latter category may include new serotypes.

Representative Results

A positive Quellung reaction occurs when type-specific antibody binds to the capsule of the pneumococcus, leading to a change in its refractive index¹⁰. When viewed under a microscope, the bacteria appear 'swollen' and more visible¹⁴. **Figure 1A** shows pneumococcal cells in HI broth. When type-specific anti-serum is added to the bacterial suspension, pneumococci appear 'swollen', refractive and more rounded (**Figure 1B**).

Too many bacterial cells added to the broth may result in aggregation of cells or a false negative reaction. **Figure 2** shows inocula of pneumococcal cells in HI broth that are 'too heavy' (**Figure 2A**) or 'too light' (**Figure 2B**).

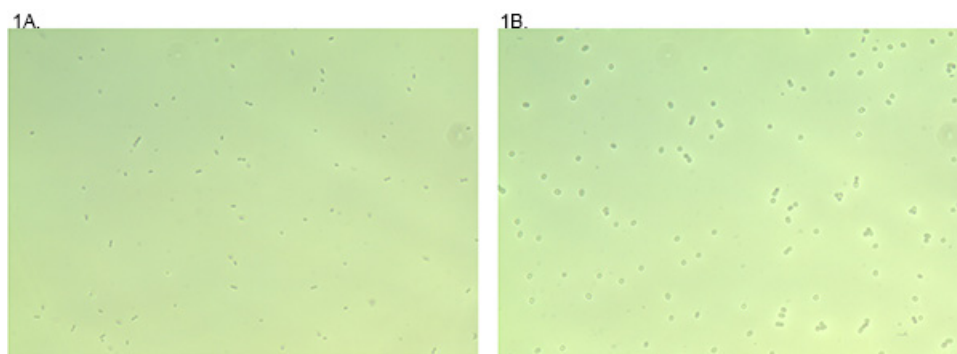


Figure 1. Negative and positive Quellung reaction. Preparations of serotype 9V pneumococcal isolate with no antiserum (1A) and with group 9 antiserum (1B), were viewed under 400X magnification. The latter shows the 'swelling' and rounding of bacterial cells typically seen in a positive Quellung reaction.

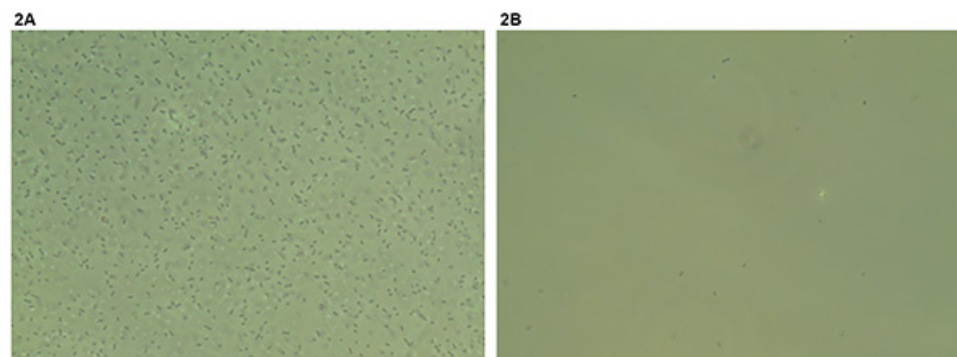


Figure 2. Too heavy and too light inocula. Preparations of pneumococcal cell suspensions that are too heavy (2A) or too light (2B), when viewed under 400X magnification.

Discussion

A critical step in this technique is ensuring a pure culture is used to prepare the suspension along with adding a suitable number of bacterial cells to the broth. It should be noted that colony growth and morphology on agar plates varies between different pneumococcal isolates. Some produce dry and rough colonies, which can be difficult to pick off with a loop and do not emulsify well in the broth. For this reason, a larger sweep of bacteria may be required when preparing the cell suspension. In any case, it is imperative that individual cells, and not aggregates, are obvious in the negative control so their relative size can be compared to the cells with antisera added. Additionally, too many bacterial cells may result in a false negative reaction, where an excess of capsular antigen may inhibit the Quellung reaction¹⁰. For this reason, it is important to create a bacterial suspension with a suitable cell density (Figure 1). The suspension may be preserved for several months by the addition of several drops of concentrated formalin. This enables easier batch testing and also sterilizes the broth reducing the risk of laboratory-acquired infections; although this must be weighed against the safety risk of handling formalin, which is toxic, corrosive, carcinogenic, sensitizing, and flammable. In addition, we have observed that some isolates show weaker reactions in the presence of formalin (data not shown).

Quellung reactions are examined using a microscope. The use of phase contrast enhances the visibility of the capsule. In some laboratories, the reaction is viewed using oil-immersion at 1,000X magnification^{14,16}. In addition, some laboratories use a counter stain (methylene blue) to further enhance the visibility of a positive reaction^{10,16,22}. The method presented here is viewed without oil-immersion and under 400X magnification^{15,17}. This method is quick, simple and relatively easy to read²³.

It is important to maintain good internal quality control procedures to ensure the antisera and testing procedures are in order. Participation in an external quality assurance program is often beneficial.

A major limitation of the Quellung reaction for serotyping of pneumococci is the high cost of the antisera required. However, this is minimized in the above protocol due to the small amount of antiserum used for each reaction. Several other methods have been described for serotyping pneumococci^{23,24}. However, Quellung remains the gold standard and the development of new methods requires standardization against the Quellung reaction²³. Using appropriate antisera, the Quellung reaction can also be utilized to identify and type other capsule producing bacteria²⁵. The Quellung reaction is instrumental in the characterization of current and emerging pneumococcal serotypes, as well as providing information on vaccine efficacy and the long-term impact of vaccination on carriage and disease.

Disclosures

The authors have nothing to disclose.

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