4 Bogdanove, E.M., Vitam. Horm. *22* (1964) 205.
5 Czygan, P.G., and Schulz, K.D., Gynaec. Invest. *3* (1972) 126.
6 Catt, K.J., and Dufau, M., A. Rev. Physiol. *39* (1977) 529.
7 Dhont, M.H., DeGezelle, H., and Vandekerckhove, D., Clin. Endocr. *5* (1976) 175.
8 Hashimoto, T., Miyai, K., Matsumoto, K., Izumi, K., and Kumahara, Y., J. clin. Endocr. Metab. *41* (1975) 1110.
9 Hsueh, A.J.W., Erickson, G.F., and Yen, S.S.C., Nature *273* (1978) 57.
10 Hsueh, A.J.W., Erickson, G.F., and Yen, SS.C., Endocrinology *104* (1979) 807.
11 Huang, E.S-R., and Miller, W.L., Endocrinology *112* (1983) 442.
12 Igarashi, M., Ibuki, Y., Kubo, H., Kamoika, J., Yokota, N., Ebara, Y., and Matsu, S., Am.J. Obstet. Gynec. *97* (1968) 120.
13 Jacobson, A., Marshall, J.R., Ross, G.T., and Cargille, C.M., Am. J. Obstet. Gynec. *102* (1968) 284.
14 Kato, J., Kobayashi, T., and Villee, C.A., Endocrinology *82* (1968) 1049.
15 Koiter, T.R., Pols-Valkhof, N., and Schuiling, G.A., Acta endocr. *92* (1979) 28.
16 Koiter, T.R., Pols-Valkhof, N., and Schuiling, G.A., Acta endocr. *96* (1981) 309.
17 Miyake, A., Tasaka, K., Sakumoto, T., Kawamura, Y., Nagahara, Y., and Aono, T., Acta endocr. *103* (1983) 289.
18 Rebar, R.H., Judd, H.L., Yen, S.S.C., Rakoff, J., Vandenberg, G., and Naftolin, F., J. clin. Invest. *57* (1976) 1320.
19 Ross, G.T., Cargille, C.M., Lipsett, M.B., Rayford, P.L., Marshall, J.R., Strott, C.A., and Rodbard, D., Rec. Progr. Horm. Res. *26* (1970) 1.
20 Roy, S., Greenblatt, R.B., and Mahesh, V.B., Acta endocr. *47* (1964) 657.
21 Santen, R.J., and Ruby, E.B., J. clin. Endocr. Metab. *48* (1979) 315.
22 Sarkar, D.K., and Fink, G., J. Endocr. *86* (1980) 511.
23 Schuiling, G.A., and Gnodde, H.P., J. Endocr. *71* (1976) 1.
24 Schuiling, G.A., and Gnodde, H.P., J. Endocr. *72* (1977) 121.
25 Schuiling, G.A., Moes H., and Koiter, T.R., Acta endocr. *106* (1984) 454.
26 Schuiling, G.A., Pols-Valkhof, N., van der Schaaf-Verdonk, G.C.J., and Koiter, T.R., J. Endocr. *103* (1984) 301.
27 Steel, R.G.D., and Torrie, J.H., Principals and procedures of statistics. McGraw-Hill, New York 1960.
28 Vaitukaitis, J.L., Bermudez, J.A., Cargille, C.M., Lipsett, M.B., and Ross, G.T., J. clin. Endocr. Metab. *32* (1971) 503.
29 Wang, C.F., and Yen, S.S.C., J. clin. Invest. *55* (1975) 201.

0014-4754/85/081060-04$1.50 + 0.20/0

Comparison of counterimmunoelectrophoresis (CIE), latex agglutination (LA) and staphylococcal coagglutination (COAG) in pneumococcal antigen detection in vitro

M.W. Rytel and M.J. Rytel

Division of Infectious Diseases, Medical College of Wisconsin, Milwaukee County Medical Complex, Milwaukee (Wisconsin 53226, USA), 26 July 1984

Summary. CIE was compared to agglutination assays employing commercial kits (Directigen, Phadebact), as well as our own LA and COAG reagents, in detection of pneumococcal capsular polysaccharide (PCP) antigens in vitro. Directigen provided the most sensitive assay. CIE was of comparable sensitivity except for PCP antigen types 7 and 14.
Key words. Pneumococcal capsular polysaccharides; antigens; antibody; counterimmunoelectrophoresis; latex agglutination; staphylococcal coagglutination.

Etiologic diagnosis in infectious diseases should be established rapidly and accurately to permit optimal therapy. Current research centers on detection of microbial antigens in body fluids. Many different techniques are being evaluated. The purpose of this study was to compare the sensitivity of counterimmunoelectrophoresis (CIE), latex agglutination (LA) and staphylococcal coagglutination (COAG) in detection of pneumococcal capsular polysaccharide (PCP) antigens in vitro. Reagents came from commercial kits or were prepared in our own laboratory.

Latex agglutination (LA). Polystyrene latex particles coated with specific antibodies are reacted with soluble antigens. Agglutination occurs when there is sufficient antigen to cause the sensitized particles to clump together and form a visible aggregate. Two preparations of latex reagents were used in this study, Directigen (Hynson, Westcott & Dunning, Baltimore, Md) and Bacto latex (Difco Laboratories, Detroit, MI) which were sensitized in our laboratory. The sensitization procedure was based on that of Kumar et al.[1] and was briefly as follows: Latex particles, 0.81 μm (undiluted, unwashed) were mixed in equal volume with Omniserum (Statens Seruminstitut, Copenhagen, Denmark), which had been diluted 1:10 in glycine buffered saline (GBS), pH 8.2, 0.1 M. This mixture was then incubated and rotated (Labquake, Labindustries, Berkeley, CA) at 37°C for 3 h, then left to stand at 4°C overnight. This sensitized preparation was diluted 1:2 with 0.1% bovine serum albumin (BSA, 0.1% in GBS) and was then ready to use. Antigen dilutions were prepared from the 14-valent pneumococcal vaccine, Pneumovax™ (Merck, Sharp and Dohme, West Point, PA) containing PCP types 1–4, 6, 8, 9, 12, 14, 19, 23, 25, 51, 56 and individual PCP types 3, 7, 9, 14 (Eli Lilly Laboratories, Indianapolis, IN). The test was run by adding one drop of latex reagent (20 μl) to one drop of antigen, mixing with a wooden applicator and then rotating on a Junior Orbit Shaker (Lab-line Instruments, Inc., Melrose Park, IL) for 5 min. Directigen test was performed according to the manufacturer's instructions.

Counterimmunoelectrophoresis (CIE). In performing CIE, a buffered diffusion medium is used into which a series of opposing wells have been punched for samples containing either antigens or antibodies. At the pH usually employed (weakly alkaline), the antigens generally will be negatively charged and will migrate towards the anode on application of electric current through the diffusion medium. Antibodies, although weakly negative or neutral, will be swept towards the cathode by a

Comparison of counterimmunoelectrophoresis (CIE), latex agglutination (LA) and staphylococcal coagglutination (COAG) in pneumococcal capsular polysaccharide (PCP) antigen detection

Technique	Least amounts of (PCP) antigen detected (μg/ml) Pneumococcal types			
	3	9	7	14
CIE	0.09	0.78	25	50
LA-Directigen	0.0055	0.045	1.56	0.39
COAG-Phadebact	0.045	0.78	6.25	1.56
COAG-Pansorbin	0.19	0.78	3.1	3.1
COAG-Cowan 1	0.78	0.78	3.1	3.1

process referred to as endosmosis. If specific for each other, antibody and antigen will meet between the two wells, combine and form an easily discernible precipitin line. The procedure employed, was as reported previously[2]. In this study, CIE was tested for its sensitivity in detecting antigens in the Pneumovax as well as individual PCP antigen types 3, 7, 9, 14.

Coagglutination (COAG). COAG is a technique for identifying specific microorganisms or their antigens using antibody coated staphylococci. Antibodies bind to protein A in staphylococcal cell wall via the Fc region, leaving the Fab region free to react with the appropriate antigen.

Three different staphylococcal preparations were tested in this study: Cowan 1 strain of *Staphylococcal aureus* (prepared in our lab) and Pansorbin *S. aureus* (Calbiochem-Behring, La Jolla, CA) both sensitized with Omniserum, and the Phadebact pneumococcal COAG kit (Pharmacia Diagnostic, Piscataway, NJ).

Preparation of *S. aureus* Cowan 1 strain was based on the method of Wasilauskas[3] and was briefly as follows: 1) The organisms were grown in two test tubes containing 5 ml of trypticase soy broth (TSB) at 37 °C for 8–12 h. 2) Using sterile technique, two 500 ml flasks, each containing approximately 250 ml of TSB, were inoculated by adding the contents of the test tubes. The flasks were then incubated at 37 °C for 18–24 h. 3) The bacteria were transferred to two 250 ml centrifuge bottles and spun down at 12,000 rpm for 10 min. Supernatant was poured off. 4) The organisms were washed five times with phosphate buffered saline (PBS) with NaN_3. 5) A 10% suspension of staphylococci (w/v) was made in 0.5% formaldehyde solution (in PBS with NaN_3) and left to stand for 3 h at room temperature. 6) The organisms were then washed three times with PBS. 7) A 10% suspension of staphylococci (w/v) was made in PBS and heated at 80 °C for 1 h in H_2O bath. 8) The organisms were finally washed three times with PBS and resuspended to a final concentration of 10% in PBS.

Staphylococci were sensitized by mixing 1 ml of the 10% suspension with 0.1 ml of Omniserum. The mixture was allowed to stand for 3 h at room temperature with occasional handshaking. Once sensitized, the organisms were harvested by centrifugation and then resuspended to 1 ml volume in PBS. A negative control was prepared following the above procedure but using normal rabbit serum instead of Omniserum.

Organisms in Pansorbin (7.1% w/v) were sensitized using the same procedure as for the Cowan 1 strain. Coagglutination tests for both the Cowan 1 strain and the Pansorbin were conducted on black agglutination plates by adding one drop (20 µl) of antigen solution to one drop of staphylococcal reagent. The drops were mixed with a wooden applicator and then the plate was rocked by hand for approximately 1 min. Finally, the plate was observed under high intensity light for signs of coagglutination.

The Phadebact Pneumococcus coagglutination kit was used according to the manufacturer's instructions.

LA test employing Directigen was the most sensitive, detecting *S. pneumoniae* vaccine antigens up to a dilution of 1:32,568. Next in order of sensitivity was COAG with Phadebact with an end point of 1:4096. CIE did not detect antigens beyond a dilution of 1:1024. Other agglutination tests were less sensitive: latex particles sensitized by us: 1:2048; both staph COAG preparations (Cowan 1 and Pansorbin) 1:512.

The results were similar when types 3 and 9 PCP antigen were assayed (table). CIE was, however, quite insensitive for types 7 and 14, which have poor anodal mobility. This has been reported previously[4] and is an important deficiency of CIE, because types 7 and 14 are of significance in pediatric infections. We conclude that, overall, latex agglutination with Directigen kit provided the more sensitive assay for detection of PCP antigens in vitro. Staphylococcal coagglutination, including reagents from the commercially available Phadebact kit, and counterimmunoelectrophoresis were less sensitive. CIE was particularly insensitive against types 7 and 14 which have poor anodal mobility. Agglutination tests are more rapid than CIE, but may require additional specimen manipulations to remove nonspecific reactants.

1 Kumar, A., Conceni, B.L., and Mankervis, G.A., Ann. clin. Lab. Sci. *10* (1980) 377.
2 Rytel, M.W., Lab. Med. *11* (1980) 655.
3 Wasilauskas, B., Lab. Med. *12* (1981) 411.
4 Coonrod, J.D., and Rytel, M.W., J. Lab. clin. Med. *81* (1973) 770.

0014-4754/85/081063-02$1.50 + 0.20/0

Endogenous copper is cytotoxic to a lymphoma in primary culture which requires thiols for growth

A. Mohindru*, J.M. Fisher and M. Rabinovitz

Laboratory of Medicinal Chemistry and Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda (Maryland 20205, USA), 26 June 1984

Summary. With the use of bathocuproine sulfonate, a copper-specific chelator as an indicator, we have demonstrated that copper ions, present as a natural medium constituent are toxic to the growth of a lymphoma in primary culture and are principally responsible for the growth requirement of mercaptoethanol and other thiols. By chelating trace copper normally present in the medium, bathocuproine sulfonate retarded the oxidation of cysteine to poorly utilized cystine, thus permitting its direct utilization by the cells for growth.

Key words. Lymphoma, primary culture; copper chelator; copper, endogenous; copper, cytotoxicity; bathocuproine sulfonate; cysteine oxidation.

A principal concern in cellular nutrition is the growth requirements of mammalian cells obtained directly from animal sources; that is, in primary culture. Unlike cells in established culture, which may have been selected as uniquely hardy specimens and then passaged continuously over generations, primary cultured cells have supplemental requirements[1]. For instance, several murine and human tumor cells in primary culture have an obligatory requirement for mercaptoethanol (ME) or other thiols, a property generally not shared by cells in established culture or by many normal cells in primary culture[2–4].

Both Ishii et al.[5] and Ohmori and Yamamoto[6] have postulated