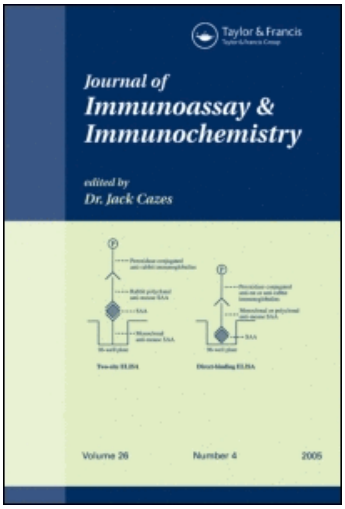


**Journal of Immunoassay and Immunochemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

# Rapid Antibody Capture Assay for Detection Of Group-A Streptococci Using Monoclonal Antibody and Colloidal Gold-Monospecific Polyvalent Antibody Conjugate

Rita Gupta[a]; G. P. Talwar[a]; S. K. Gupta[a]
[a] Gamete Antigen Laboratory, National Institute of Immunology, New Delhi, INDIA

**To cite this Article** Gupta, Rita , Talwar, G. P. and Gupta, S. K.(1992) 'Rapid Antibody Capture Assay for Detection Of Group-A Streptococci Using Monoclonal Antibody and Colloidal Gold-Monospecific Polyvalent Antibody Conjugate', Journal of Immunoassay and Immunochemistry, 13: 3, 441 — 455
**To link to this Article: DOI:** 10.1080/15321819208021242
**URL:** http://dx.doi.org/10.1080/15321819208021242

JOURNAL OF IMMUNOASSAY, 13(3), 441-455 (1992)

# RAPID ANTIBODY CAPTURE ASSAY FOR DETECTION OF GROUP-A STREPTOCOCCI USING MONOCLONAL ANTIBODY AND COLLOIDAL GOLD-MONOSPECIFIC POLYVALENT ANTIBODY CONJUGATE

Rita Gupta, G.P.Talwar and S.K.Gupta
Gamete Antigen Laboratory, National Institute of Immunology, Shahid Jeet Singh Marg, New Delhi-110067, INDIA.



## ABSTRACT

A rapid one step, sensitive and specific antibody capture assay for detection of group-A streptococci from the throat swabs of children is described. Monoclonal antibody either MA-106 or MA-107 specific for group-A streptococci polysaccharide (APS) was used as the capture antibody on nitrocellulose paper and rabbit monospecific polyvalent antibody conjugated with colloidal gold to detect the presence of antigen. The lower detection limit of this assay is 15.6ng APS/ml. The assay is specific for APS and failed to recognize polysaccharides obtained from group-B,-C,-G streptococci as well as Staphylococcus aureus. Antigen extracted from throat swabs of children who were positive for β-hemolytic plaques (other than group-A streptococci) as seen on blood agar culture gave negative readings, thereby confirming the specificity of the assay for APS. (**KEY WORDS:** Group-A streptococci, Antibody capture assay, Monoclonal antibody, Antibody-colloidal gold conjugate).

## INTRODUCTION

Post infective sequelae of repetitive infection with streptococci-A in children is recognized to lead to rheumatic fever (RF)/rheumatic heart diseases (RHD) and acute glomerulonephritis. A conservative estimate is

that 2-3 million children suffer every year from RF in India alone. To reduce the incidence of RF/RHD it will be desirable to identify all streptococcal-A throat infections and treat the infected individuals adequately. Diagnosis of group-A streptococcal pharyngitis relies mainly on the culture of the organisms on blood agar plates followed by identification of $\beta$-hemolytic plaques by bacitracin sensitivity. This procedure requires 48 hr and a microbiology laboratory. However, rapid antigen detection assays based on precipitation, coagglutination, latex agglutination and enzyme linked immunosorbent assay (ELISA) have been reported (1-6). Agglutination assays require experience to read the result and also careful processing of samples to avoid non-specific agglutination. In this communication, we report a simple one step antibody capture assay which takes 5min, and can be read visually by the appearance of a purple dot. The assay employs monoclonal antibody dotted on nitrocellulose strip which acts as a capture antibody. When group-A polysaccharide and second antibody colloidal gold conjugate are mixed, the immune complexes thus formed are allowed to move up by capillary action through the nitrocellulose membrane, which are retained by the capture antibody. Deposition of this complex appears in the form of purple dot due to concentration of colloidal gold. In absence of antigen, no antigen-antibody complex is formed and thus

second antibody colloidal gold conjugate is not trapped by capture antibody and hence no colored dot.

## MATERIALS AND METHODS

### Bacterial Strains

Streptococci strains group-A (J17 A4), group-B (090 R), group-C (Chestle), group-G (Valente) and Staphylococcus aureus were obtained from Dr. K. Prakash, WHO Collaborating Centre for Reference and Training in Streptococcal Diseases, Department of Microbiology, Lady Harding Medical College, New Delhi. The purity of these bacteria were checked on blood agar plates. For bulk culture, bacteria were grown in Todd Hewett broth liquid medium for 18 hr at $37^0$C followed by inactivation at $56^0$C for 30 min. Bacteria were harvested by centrifugation at 1500 g for 15min and washed subsequently 5x with sterile PBS (50mM phosphate, 150mM NaCl, pH 7.4 ).



### Antigen Preparation

Polysaccharides were isolated and purified from bacteria by nitrous acid extraction (1). 20mg of group-A streptococci polysaccharide (APS) was dissolved in 2ml of dioxane containing 2mg/ml carbonyldiimidazole and continuously mixed for 2hr at room temperature. Activated polysaccharide was precipitated by addition of 10ml of acetone and the pellet washed twice with 5ml acetone. The pellet was dissolved in 2ml

of 0.1M borate buffer, pH 8.6, 50mg bovine serum albumin (BSA) added and mixed overnight at $4^0$C. The conjugate was dialyzed against 0.9% NaCl with 4 changes at $4^0$C and stored at $-20^0$C.

Production Of Polyclonal Antibodies

New Zealand white rabbits were immunized intradermally (ID) at multiple sites with 100$\mu$g APS-BSA emulsified with incomplete Freund's adjuvant (IFA). After 4 weeks, animals were boosted with 50$\mu$g APS-BSA intramuscularly (IM). Bleeds were checked for antibody titer against trypsinized streptococci-A and APS in ELISA as described later in the section. Animals were subsequently boosted at the time of decline in antibody titres. Immune serum was rendered monospecific by passing through a column packed with cyanogen bromide activated Sepharose-4B coupled to BSA, pre-equilibrated with 10mM phosphate buffer containing 150mM NaCl, pH 7.4. The column was eluted with the same buffer. Fractions containing unbound proteins from Sepharose-4B-BSA column were pooled and loaded on a column packed with Sephadex G-25 (Sigma Chemical Co. St.Louis, USA) mixed with whole bacterial suspension (4:1 ratio by volume). The bacterial suspension consisted of an equal ammount of streptococci-B,-C,-G and Staphylococcus aureus. The column was eluted with 150mM NaCl. Unbound eluted fractions from the bacterial column were pooled,

immunoglobulins precipitated by ammonium sulphate (40% saturation), dialysed against 50mM phosphate buffer (pH 7.4). The antibodies were further purified on a DEAE-A50 column. Unbound eluted fractions were pooled, and concentrated by Amicon membrane to the original volume of the sera taken for purification.

Generation Of Monoclonal Antibodies

Inbred BALB/c mice reared at the Institute Small Animal Facility were immunized subcutaneously with $2x10^7$ group-A streptococci bacteria emulsified with incomplete Freund's adjuvant (IFA) and boosted 4 weeks later intraperitoneally with $1x10^7$ bacteria in IFA. After 2 months, a final injection of $2x10^7$ bacteria was given intravenously and the spleen removed after 4 days. Hybrid cell clones were developed by fusing splenocytes obtained from immunized mice with SP2/O Ag 1.4 mouse myeloma cells as described elsewhere (7). Wells positive for the growth of hybrid cells were screened for anti-streptococcal-A antibodies by ELISA and hybrid cells from the identified positive wells were cloned by limiting dilution and subcloned repeatedly to obtain stable cell lines secreting monoclonal antibodies (MCAs). Hybrid cells were grown in the peritoneal cavity of Pristane (Aldrich chemical Co.,Milwaukee,USA.)- primed BALB/c mice as ascites.

Ascites fluid tapped from the peritoneal cavity was made cell free by centrifugation at 8oo x g for 15min at $4^0$C. Immunoglobulins of IgM isotype, were precipitated from ascites by extensive dialysis against water at $4^0$C, washed once with water and dissolved in PBS. MCAs were subsequently purified on Cl-6B column.

Characterization Of Monoclonal And Polyclonal Antibodies

Rabbit polyclonal and mouse MCAs were screened for their reactivity with different strains of bacteria by an ELISA. Briefly, 96 well polyvinyl microtitration plates were coated with whole bacteria ($15x10^6$ per well in 50mM carbonate buffer, pH 9) or trypsinized streptococci-A (1mg trypsin/ml, overnight at room temperature) and dried at $37^0$C. To determine the reactivity of antibodies with APS, due to its poor adsorption on microtitration plate, APS was conjugated to poly-L-lysine (molecular weight 40,000;Sigma Chemical Co., St.Louis, Mo.) as described previously (8) and microtitration plates were coated with conjugate (1$\mu$g APS equivalent per well), for 1 hr at $37^0$C followed by overnight at $4^0$C. After coating, plates were washed with PBS containing 0.05% Tween-20 (PBST) and nonspecific binding sites blocked by adding 200$\mu$l per well of 1% polyvinyl alcohol in PBST and incubating the plates at $37^0$C for 1 hr. Plates were subsequently washed with PBST and incubated

with 100μl of either polyclonal or MCAs at appropriate dilutions. In case of monoclonal antibodies, control wells had PBST, culture supernatant from SP2/O-Ag 1.4 myeloma cells or antibody from unrelated mouse hybrid cell clones whereas for rabbit polyclonal antibodies, PBST and pre-immune sera served as respective controls. Plates were incubated for one hr at $37^0$C and subsequently washed with PBST. In the case of MCA, rabbit anti-mouse immunoglobulin coupled to horseradish peroxidase (HRP, DAKO corporation, Santa Barbara, Ca, USA), for rabbit polyclonal antibodies, protein-A-HRP (Zymed, Ca, USA) were added at the appropriate dilutions and plates incubated for 1hr at $37^0$C. After incubation the plates were washed with PBST, enzyme activity determined by adding 100μl of freshly prepared substrate solution (0.5mg of o-phenylenediamine dissolved in 1ml of 50mM citrate buffer, pH 5.6 containing 0.03 % hydrogen peroxide). The reaction was stopped by adding 50μl of 5N $H_2SO_4$, and plates were read at 490nm in an automatic ELISA reader.



**Antibody Capture Assay**

i) Antibody Colloidal Gold Conjugation

Colloidal gold sol was prepared from gold chloride (HAuCl) (Amrésco, Ohio, USA ) using 1 % sodium citrate as described previously (9) . Briefly, 50 ml of

0.02% HAuCl in water was boiled. As soon as it began to boil, 1.2 ml of 1% sodium citrate was added with constant stirring. Color changed from grey to blue and purple violet within 45 -60 seconds. After the color change, the heat was turned off, the solution stirred for 2-3 min, and allowed to cool at room temperature. Colloidal gold sol was scaned between 450-700nm and the batch having $\lambda$ max between 525-535nm was subsequently used for preparing the conjugate with antibody. To 1ml of colloidal gold sol in a glass tube, 6-10$\mu$l of 1% $K_2CO_3$ ( to adjust the pH of gold sol to 6.5) was added followed by 6$\mu$g of monospecific polyvalent antibody and vortexed. It was incubated for 2-5 min at room temperature followed by addition of 6$\mu$l of 20% BSA ( final concentration approximately 0.1%), and the contents were transferred in a Eppendorf tube and centrifuged for 5min in a Eppendorff centrifuge at 5000 RPM. The supernatant except 50$\mu$l was discarded and the pellet resuspended in it.



ii) Assay

Throat swabs in duplicate were collected from the schoolgoing children (low income group) aged 3-15 years. From one of the swabs kept in a tube, the polysaccharide was extracted by adding 200$\mu$l each of 1M $NaNO_2$ and 1N acetic acid and the extract neutralized

subsequently (indicated by the change in colour of phenol red indicator) by adding 0.4 M $Na_2CO_3$. Duplicate swabs were employed for streaking the organisms on blood agar plate [2% Nutrient Agar (Difco Laboratories, Michigan USA) containing 7% defibrinated sheep blood]. The plates were incubated overnight at $37^0C$ and checked for $\beta$-hemolytic colonies. Single $\beta$-hemolytic colony from the plates was streaked on a fresh blood agar plate. Two discs (whatman No. 1) of 6mm diameter containing 0.7 units of bacitracin per disc, were placed in each plate and bacteria allowed to grow for 24 hrs at $37^0C$. The inhibition in the growth of bacteria as judjed by no hemolysis was indicative of group-A streptococci.

For antibody capture assay 5$\mu$g MCA (MA-106/107) was dotted on nitrocellulose (7.5cmx5mm, 5$\mu$m pore size) strips and dried at room temperature. To a microtitration plate, antibody colloidal gold conjugate 2-5$\mu$l/well was added followed by 10$\mu$l of 10 % alkali-treated casein and 40$\mu$l of sample (known amount of APS or polysaccharide extracted from the throat swab) and the contents mixed. An antibody dotted nitrocellulose strip was put into the well and the solution allowed to rise by capillary action. The samples positive for the presence of streptococci-A infection showed a purple violet dot as compared to its absence in the negative samples.

## RESULTS

Two hybrid cell clones secreting MCAs were stabilized by fusing splenocytes from mouse immunized with group-A streptococci with SP2/O Ag 1.4 mouse myeloma cells. Reactivity of these two antibodies with APS, streptococci-A (trypsinized as well as nontrypsinized), streptococci-B, -C, -G, Staphylococcus aureus, E.coli and S.typhi is given in Table 1. Both antibodies were of IgM isotype. They gave a strong reaction with APS, and trypsinized and nontrypsinized streptococci-A, but failed to recognize the other strains of streptococci as well as other gram negative bacteria. Both MCAs reacted equally well with trypsinized and nontrypsinized streptococci-A.

The properties of the rabbit monospecific polyvalent antibodies generated against APS-BSA is given in Fig.1. Antibodies at a concentration of 2μg/ml gave a strong reaction with trypsinized as well as nontrypsinized streptococci-A including APS. Antibodies even at 25 times higher concentration failed to recognize streptococci-B,-C,-G and Staphylococcus aureus.

The antibody capture assay as described in Materials and Methods using MA-106 as capture and monospecific polyvalent antibody-colloidal gold conjugate for revealing gave a positive dot with 15.6ng APS/ml. The assay did not recognize polysaccharide from streptococci-B, -C, -G and Staph.aureus. Out of 150

TABLE 1.

**Reactivity Pattern Of Murine Anti-Streptococci-A Monoclonal Antibodies In ELISA**

| MCA No. | A 490 with | | | | | | |
|---|---|---|---|---|---|---|---|
| | Streptococci-A | | | Streptococci- | | | Staph. aureus, |
| | APS | Tryp. | Nontryp. | -B | -C | -G | E.coli, S.typhi |
| 106 | 0.91 | 2.04 | 2.91 | 0 | 0 | 0 | 0 |
| 107 | 1.20 | 2.12 | 2.39 | 0 | 0 | 0 | 0 |

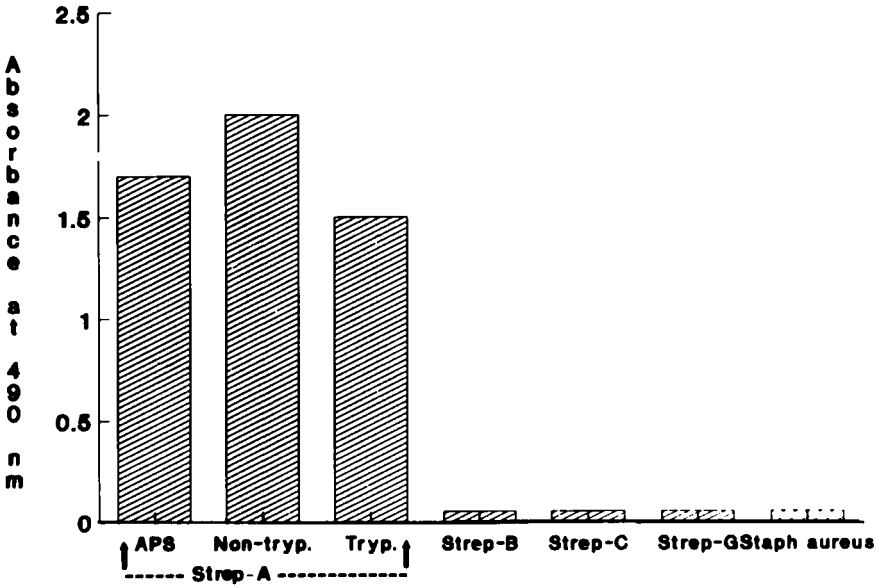


FIGURE 1. Reactivity pattern in ELISA with APS, streptococci-A (trypsinized & nontrypsinized), strep.-B, -C ,-G and staph.aureus of purified monospecific polyvalent anti-streptococcus-A antibodies raised in rabbits against APS-BSA conjugate. The antibody was used at a concentration of 2μg/ml for testing the reactivity with APS & streptococci-A and at a concentration of 50μg/ml with other bacteria.

TABLE 2.

**Detection Of Streptococci-A From Duplicate Throat Swabs Of Children By Antibody Capture Assay And Blood Agar Culture**

| No. of Children 3-15 yrs | Positive By Antibody-Capture Assay | Positive by Blood Agar culture | |
|---|---|---|---|
| | | β-hemolytic non group-A | β-hemolytic group-A |
| 150 | 3 | 27 | 3 |



throat swabs collected at random from children in the age group of 3-15 years, only 3 were positive in this assay. These were also the only samples positive by blood agar culture for streptococci-A (Table 2). Twenty seven samples grew β- hemolytic streptococci which were identified as being other than group-A on the basis of bacitracin sensitivity and were also found to be negative in the antibody capture assay, thereby indicating the specificity of the test.

## DISCUSSION

The culture of throat swab on blood agar plates followed by further identification of β-hemolytic colonies by bacitracin sensitivity is of high predictive value for the physician and thus avoids indiscriminate use of antibiotics(10). However, a

physician confronted with an acute case of throat infection would like to define the etiological agent rapidly. The antibody capture assay described here is one such rapid test for diagnosis of group-A streptococci. The assay is simple, easy to perform and the results can be obtained in less then 10 minutes after collecting the swabs (including extraction of the antigen from throat swab). In contrast to enzyme immunoassay the present assay is a one step assay. It does not require seperation of free antibody from antigen antibody complex and subsequent quantitation of enzyme activity by adding appropriate substrate. The assay is highly specific for group-A by virtue of the fact that it employs a monoclonal antibody (MA-106 or MA- 107). This is further confirmed by the fact that 27 samples which grew $\beta$- hemolytic plaque other then group-A as determined by bacitracin sensitivity test, were found negative by this assay. However, the number of positive cases (three) for group-A streptococci studied from throat swabs collected randomly is insufficient to define the sensitivity of the test. If the assay performs acceptably in a larger series, it has potential for a rapid diagnostic kit which can be used by school teachers or parents. The assay has the requisite sensitivity, comparable or even better than the assays reported previously (1-6).

## ACKNOWLEDGEMENTS

We gratefully acknowledge the help received from the management of the schools run by Mobile Creches for permission to collect throat swabs from the children and Dr. Harini Bagavant for her critical suggestions. R.G. was supported by a fellowship from the Talwar Research Foundation.

Address correspondence to:

Dr. S. K. Gupta,
Gamete Antigen Lab,
National Institute Of Immunology,
JNU Campus,
Shahid Jeet Singh Marg,
New Delhi-110067, INDIA



## REFERENCES

1. Facklam,R.R. Streptococci and Aerococci,In Lennette E.H., Balows A., Husler W.J., Jr. and Traunt,J.P.eds. Manual of Clinical Microbiology Third Edition. American Society for Microbiology, Washington, D.C.1980;88-110.

2. Otero,J.R.,Reyes,S. and Noriega,A.R.Rapid Diagnosis of Group-A Streptococcal Antigen Extracted Directly from Swabs by an Enzymatic Procedure and used to Detect Pharyngitis.J.Clin.Microbiol.1983;18:318-28

3. Manasse,R.J. Evaluation of the Pacific Bioteck CARDS STREP A Test for Detecting Group A Streptococci from Cases of Pharyngitis. J.Clin. Microbiol.1989;27:1657-8.

4. Dobkin,D. and Shulman,S.T. Evaluation of an ELISA for Group A Streptococcal Antigen for Diagnosis of Pharyngitis. J. Pediatr. 1987;110:566-89.

5. Kellogg,J.A., Bankert,D.A., Schonauer,T.D., Landis, R.C., Nussbaum,A.S. and Levisk,J.S. Detection of Group-A Streptococci by Aerobic Culture and a New Simplified Immunoassay in Three Pediatric Practices and Hospital Laboratory. J. Clin.Lab. Anal. 1991;5:367-71.

6. Edwards,E.A, Phillips,I.A, and Suiter,W.C.Diagnosis of Group-A Streptococcal Infection Directly from Throat Secretions. J.Clinical Microbiol. 1982; 15: 481-3.

7. Gupta,S.K. and Talwar,G.P. Hybridomas as Source of Antibodies for Immunoassay-Monoclonal Against LHRH and Their Detection by EIA. In Talwar, G.P., ed. Non-isotypic Immunoassays and Their Applications. Vikas Publishing House, India 1983;127-35.

8. Barret,D.J., Triggiani,M. and Ayoub,E.M. Assay of Antibody to Group A Streptococcal Carbohydrate By Enzyme-Linked Immunosorbant Assay.J.Clin.Microbiol. 1983; 18: 622-7.

9. Martin,J.M.C., Paques, M., Groat, T.A.M.V. and Beuvery, E.C. Characterization of Antibody Labelled Colloidal Gold Particle and Their Applicability in a Sol Particle Immunoassay ( SPIA ). J. Immunoassay 1990; 11:31-47.

10. Stollerman, G.H. Global Changes in Group A Streptococcal Diseases and Strategies for Their Prevention. Adv. Intern. Med. 1982; 27: 373-406.