J. Hyg., Camb. (1981), **86**, 315
Printed in Great Britain

Rubella serology: a comparison of four methods for exclusion of non-specific serum inhibitors

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(*Received* 13 *October* 1980)

SUMMARY

The ability of the pyrogenic silica Aerosil 380^R to exclude non-specific serum inhibitors (NSI) of rubella virus haemagglutination was evaluated. The developed procedure was compared with the kaolin, heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$ methods.

Aerosil and kaolin were found superior for the elimination of non-specific inhibitors and high density lipoproteins (HDL). The other methods left NSI and HDL in a majority of the sera, occasionally in high titres. Aerosil seemed to be somewhat more efficient than kaolin in NSI and HDL exclusion. The Aerosil method offers the opportunity to detect sera with rubella antibody titres < 10. Among eight such sera, six were shown to contain rubella antibodies, while two were false positives.

INTRODUCTION

Although new and promising methods for detection of antibodies to rubella virus, such as enzyme-immuno-assay (EIA; Voller & Bidwell, 1975), radio-immuno-assay (RIA; Kalimo *et al.* 1976) and hemolysis-in-gel (HIG; Skaug, Ørstravik & Ulstrup, 1975) have been developed recently, the haemagglutination inhibition (HI) test will no doubt remain a standard method for diagnosis, sero-epidemiology and immuno-surveillance.

The main problem of HI is the presence of antibody-mimicking, non-specific lipoprotein inhibitors in sera. These are found within all the three main classes of serum lipoproteins (Haukenes, 1973; Blom & Haukenes, 1974; Shortridge & Ho, 1974; Shortridge & Ho, 1976; Ellis & Campbell, 1977; Ho & Shortridge, 1977 and Steinmann, 1977*a*). The low-density lipoproteins (LDL), also known as β-lipoproteins, usually contain the strongest activity.

Kaolin treatment (Halonen, Ryan & Stewart, 1967) and precipitation with polyvalent anion-divalent cation combinations (Cooper *et al.* 1969; Liebhaber, 1970) have been used most commonly to remove non-specific inhibitors. These methods are known to fail in some instances (Schmidt & Lennette, 1970; Haukenes & Blom, 1975 and Steinmann, 1977*a* and *b*). This may lead to doubt concerning the specificity of low-rubella HI titres in sera.

The well characterized and standardized colloidal, pyrogenic silica Aerosil 380^{R} has been used to produce storage stable, hepatitis-free sera for transfusion (Stephan, 1971). It has been employed in production of HBsAg subtype-specific antisera (Siebke, Kjeldsberg & Traavik, 1972; Traavik, 1975) and arbovirus haemagglutinating (HA) antigens (Traavik, 1977). The ability of Aerosil to absorb lipoproteins was utilized in these instances and the silica has been shown to be an effective absorbent of serum LDL (Siebke *et al.* 1972).

This paper describes Aerosil treatment of sera before rubella HI testing, and compares it with three commonly used methods for pretreatment.

MATERIALS AND METHODS

Sera

Sera from 40 healthy young men were taken on their arrival for military recruit training. Sera from 37 pregnant women, remitted for the legally required syphilis antibody screening, were handed over from the serological section of the Department of Microbiology. Sera from 48 female staff members were received from the Department of Paediatrics at the University clinic.

After the initial evaluation of the Aerosil method, 612 patient sera, remitted for rubella diagnostics or serological control, were tested in parallel for rubella HI antibodies after pretreatment with kaolin and Aerosil.

Kaolin treament

The sera were treated with 25% kaolin in borate saline pH 9·0 (Behringwerke, batch no. 62) for 20 min at room temperature (Halonen *et al.* 1967). The serum dilution after treatment was 1 in 10.

Heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$ precipitation

The treatments were performed with commercial kits (Flow Laboratories) produced according to the recommendations of the Centre of Disease Control, Atlanta, Georgia (U.S. Public Health Service, 1975). The instructions of the manufacturers were followed carefully. Serum dilution after treatment was 1 in 4 for both methods.

Aerosil treatment

Aerosil 380 is a colloidal, pyrogenic silica, which, according to the manufacturers (Degussa, Frankfurt am Main), consists of aggregated 7 nm primary particles with a surface area of approximately 380 m^2 per g. We have used, and compared four different batches during these studies (control numbers S 313119 OC, S 314119 OC, S 315119 OC, S 316119 OC). The preparations were gifts from the manufacturers.

The dry silica powder was added to serum at a concentration of 20 mg per ml (Siebke *et al.* 1972; Traavik, 1977). Sera were treated undiluted or at a 1 in 5 dilution in PBS, pH 7·4. Earlier studies (Siebke *et al.* 1972; Traavik, 1977) indicated less complete lipoprotein adsorption at pH 9·0. The silica was thoroughly

suspended by mechanical shaking. The tubes were placed horizontally in a water-bath with constant shaking for 30 or 60 min at 37 or 45 °C. Finally the suspensions were centrifuged at 5000 rev./min for 15 min. The supernatants were pipetted off and used in the rubella HI test.

Rubella HI test

Following the procedures for lipoprotein removal, sera or serum fractions were absorbed with the erythrocytes which were to be used in the HI test, either cells from newly hatched chicks or formalinized sheep erythrocytes. Red blood cells were delivered by the Department of Laboratory Animals, National Institute of Public Health, Oslo.

HI tests were performed with microtitration equipment. Sera treated with Aerosil or kaolin were tested according to the procedure described by Halonen *et al.* (1967). Sera treated with heparin/$MnCl_2$ or dextran sulphate/$CaCl_2$ were tested according to the recommendations of CDC (U.S. Public Health Service, 1975). The HA antigen was a Tween-ether extract of rubella virus grown in BHK 21/C 13 cells (Behringwerke). Antigen and serum or serum fractions were incubated at 4 °C overnight before the addition of erythrocytes.

Flotation centrifugation

Separation of lipoproteins and immunoglobulins based on density in NaBr solutions, was performed as described by Blom & Haukenes (1974) employing a Beckman L3-50 ultracentrifuge with rotor SW 50·1. NaBr interfered with the settling of chick erythrocytes, causing non-specific haemagglutination. This problem could be avoided by dialysing all fractions. We found, however, that the settling of formalinized sheep erythrocytes was not influenced by NaBr. The HA titre of the antigen was lower with these cells, but HI titres of selected sera were identical in tests performed with chick and formalinized sheep red blood cells.

Determination of immunoglobulins and lipoproteins

Concentrations of IgG, IgM, IgA and β-lipoprotein were determined by the Mancini technique employing gel diffusion plates and reagent standards from Behringwerke. Residual and original concentrations of α_1-(high density lipoproteins, HDL) and β-lipoproteins were also compared by titrations in closed hexagon immunodiffusion (Traavik, Siebke & Kjeldsberg, 1972), using specific antisera from Behringwerke.

Haemolysis-in-gel (HIG)

HIG was performed as described by Skaug *et al.* (1975). Briefly, 0·3 ml 5% chick erythrocytes in veronal buffer pH 7·2, sensitized with rubella HA antigen, were mixed with 3 ml 1·5% melted agarose in veronal buffer at 45 °C. The mixture was poured into immunodiffusion plates (Hyland). After gelation, 2·5 mm wells were punched out. Sera were inactivated at 56 °C for 45 min and absorbed with erythroyctes. Wells were filled with 5 μl. After the sera had diffused out, the wells were sealed with agarose. The plates were flooded with 0·2 ml guinea pig comple-

ment which had been absorbed with erythrocytes. After 18 hours at 4 °C, the immunoplates were incubated 1–2 hours at 37 °C and the diameters of haemolysis zones were measured by a graduated eyepiece. Immunoplates with non-sensitized chick red cells were used as controls for non-specific haemolysis. HIG detects specific rubella IgG, but not IgM (Strannegård, Grillner & Lindberg, 1975). Some sera were concentrated 4–5 times by Lyphogel (Gelman). A standard curve for the correlation of HI titres and HIG zone diameters was established by careful dilution of two sera with pre-determined HI titres.

RESULTS

Conditions for Aerosil treatment

Indications had been given that incubation time and temperature might affect the efficiency of lipoprotein absorption with Aerosil (Siebke *et al.* 1972; Stephan, 1971). The effect of incubation at 37 °C and 45 °C for 30 and 60 min was examined with 20 randomly selected human sera, diluted 1 in 5. Non-specific inhibitors were not detected by flotation centrifugation irrespective of treatment conditions, and all rubella HI titres were practically identical (i.e. within one dilution). Aerosil treatment was conducted at 45 °C for 30 min throughout the subsequent studies.

Different batches of Aerosil 380

Twenty randomly selected undiluted patient sera were titrated for rubella HI antibodies after treatment with Aerosil of four different batches. After flotation centrifugation, non-specific inhibitors were detected in the same two sera in titres of 2–4 irrespective of Aerosil batch employed. The HI titres differed by no more than one dilution step for each serum. For 17 of the sera the titres were identical.

Preliminary testing of Aerosil for use in rubella HI

The 37 sera from pregnant women were titrated for HI antibodies untreated and after Aerosil treatment. The results are shown in Table 1. Treatment reduced titres considerably in most of the sera. The origin of the HI activities was examined by flotation centrifugation. Activity remained in the lipoprotein (top) fraction, at a dilution corresponding to 1 in 8 with one of the sera (no. 9). The top fraction titre of this serum was 512-1024 before treatment, and the bottom fraction (immunoglobulin) titre was 128–256 both before and after Aerosil treatment. By immuno-diffusion no α_1- or β-lipoproteins were detected in the post-treatment top fraction of this serum, but α_1-lipoprotein was detected in the post-treatment top fraction of another serum (no. 29). However, no associated HI activity was found in this fraction.

Preliminary comparison of Aerosil and kaolin treatment

Parallel HI titrations were performed after kaolin (starting dilution 1 in 10) and Aerosil treatment (starting dilution 1 in 5) of the 40 sera from military recruits. The results are shown in Fig. 1. The titre differences were within one dilution step for 36 of the sera. Identical titres were obtained for 21 sera, including 3 with

Table 1. *Rubella HI titres of sera from 37 pregnant women before and after lipoprotein absorption by colloidal silica gel (Aerosil)*

	HI titre			HI titre	
Serum	Untreated	Absorbed	Serum	Untreated	Absorbed
1	1024	16	20	64	32
2	1024	64	21	2048	256
3	2048	128	22	1024	32
4	64	32	23	64	8
5	512	< 2	24	512	64
6	1024	256	25	2048	128
7	512	64	26	1024	256
8	4096	128	27	512	128
9	2048	128	28	256	256
10	2048	64	29	1024	512
11	512	32	30	512	128
12	256	64	31	2048	256
13	512	32	32	1024	64
14	1024	256	33	512	32
15	256	128	34	2048	256
16	1024	256	35	512	64
17	1024	256	36	1024	256
18	2048	256	37	512	64
19	1024	512			

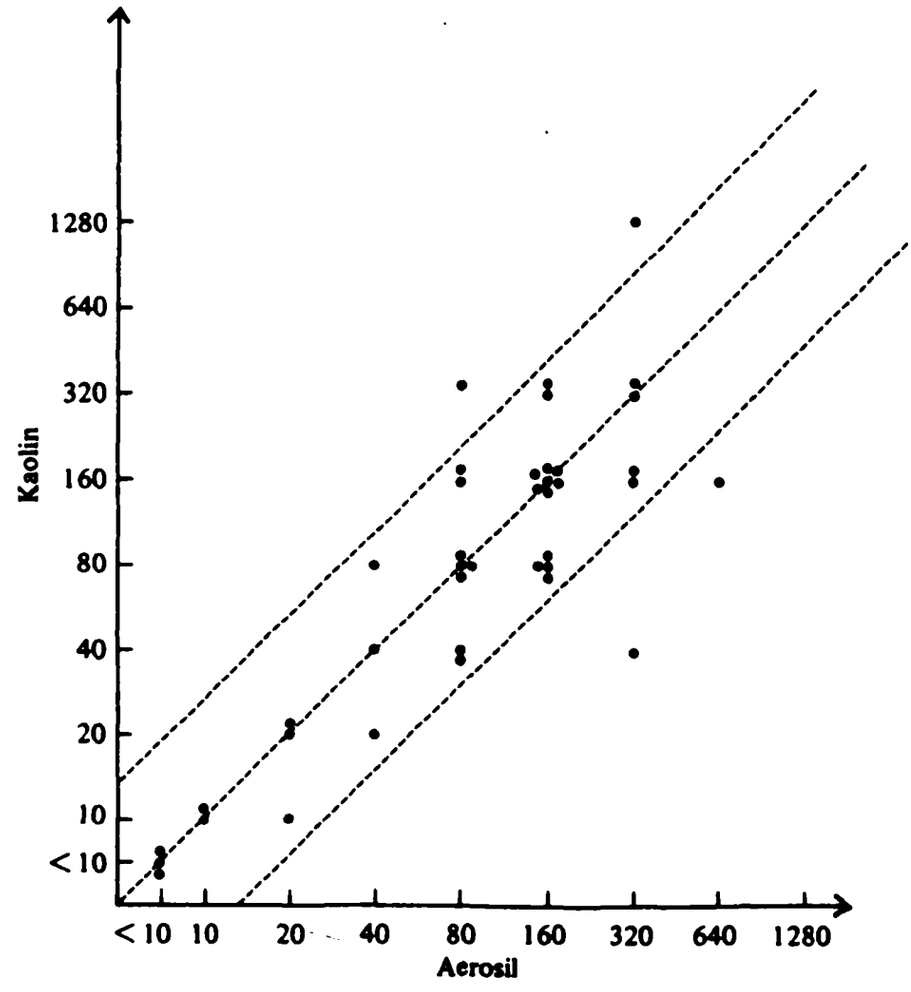

Fig. 1. Correlation between rubella HI titres obtained after kaolin and Aerosil treatment of sera from 40 healthy military recruits.

https://doi.org/10.1017/S0022172400069060 Published online by Cambridge University Press

titres < 10. For 4 sera the titres differed significantly (2–3 dilution steps). This was reproduced in repeat titrations. After retreatment, the differences were eliminated. In HIG all these sera gave haemolysis zones with diameters corresponding to identical HI titres following kaolin and Aerosil treatment. Insufficient amounts of sera precluded further investigations.

Comparison of kaolin, Aerosil, heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$

The experiments were performed with the 48 sera from the staff members of the Department of Paediatrics. Starting dilutions were 1 in 5 for Aerosil, 1 in 10 for kaolin and 1 in 4 for heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$.

Rubella HI titres. The results are accumulated in Table 2. The geometric mean titres (gmts) were 300 after heparin/$MnCl_2$, 291 after dextran sulphate, 220 after kaolin and 152 after Aerosil treatment.

A marked difference was seen between titres after Aerosil or kaolin treatment on the one hand and after dextran sulphate/$CaCl_2$ or heparin/$MnCl_2$ on the other.

All sera were fractionated by flotation centrifugation untreated and after treatment with the four different methods. The rubella HI titres of the bottom (immunoglobulin) fractions are shown in Table 2, columns 5–9. The gmt in the bottom fractions of untreated sera was 280. The bottom fractions of pretreated sera had gmts of 218 for kaolin, 186 for Aerosil, 184 for heparin/$MnCl_2$ and 178 for dextran/$CaCl_2$. Two of the 48 sera (1049 and 1088) were negative by all four methods. Lack of HI activity in the bottom fractions, both untreated and treated, confirmed these findings. A third serum (1067) was negative after kaolin, but positive at low titre after the other treatments. HI activity was found in all bottom fractions for this serum.

Elimination of non-specific inhibitors. The top (lipoprotein) fractions of the 48 sera were examined for rubella HI activity. The results are shown in columns 10–14 of Table 2. Residual inhibitory activity was found in 41 sera following heparin/$MnCl_2$ treatment and in 33 sera after dextran sulphate/$CaCl_2$ treatment. Inhibitors remained in eight sera after Aerosil and in four sera after kaolin treatment. In this connection the initial dilution factors should be kept in mind. The gmts, based on all sera, of residual non-specific HI activity were 11·5 for heparin/$MnCl_2$, 6·5 for dextran sulphate/$CaCl_2$ and 1·3 for both kaolin and Aerosil. Gmts based on positive fractions were 17·4 for kaolin and 6·5 for Aerosil. Sera with non-specific inhibitors after kaolin or Aerosil treatment showed activity at an equal or higher level after heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$ treatment.

Elimination of α_1 and β-lipoproteins. By the Mancini technique β-lipoprotein was undetectable in all 48 sera irrespective of pre-treatment. Twenty sera were examined for residual lipoproteins by closed hexagon immunodiffusion. Again β-lipoproteins were not detected in any serum irrespective of pre-treatment.

In 19 sera α_1-lipoproteins were detected after dextran sulphate/$CaCl_2$ treatment, in 17 after heparin/$MnCl_2$ treatment, in eight after Aerosil and in six after kaolin treatment (Table 3). Reactions were strongest for dextran sulphate/$CaCl_2$-treated sera. Heparin/$MnCl_2$-treated sera gave stronger reactions than Aerosil or kaolin-treated sera. At a dilution of 1 in 10, i.e. corresponding to the kaolin dilution, only

Table 2. *Rubella HI titres in sera and in immunoglobulin and lipoprotein fractions of sera after treatment with kaolin, Aerosil, dextran sulphate and heparin*

	Rubella HI titre of													
	Unfractionated serum				Bottom fraction[1]					Top fraction[1]				
Serum	K[2]	A[3]	D[4]	H[5]	U[6]	K	A	D	H	U	K	A	D	H
1048	80	80	64	128	40	40	40	32	64	320	< 10	< 5	4	16
1049	< 10	< 5	< 4	< 4	< 5	< 10	< 5	< 4	< 4	320	< 10	< 5	< 4	< 4
1050	640	320	512	512	320	640	320	256	512	320	< 10	< 5	16	16
1051	2560	2560	2048	2048	1280	1280	1280	1024	1024	640	< 10	5	32	64
1052	320	80	128	256	320	160	160	64	128	320	< 10	< 5	4	16
1053	320	320	512	512	320	160	160	512	128	640	< 10	< 5	16	32
1054	320	320	256	512	320	320	320	256	256	320	< 10	< 5	16	16
1055	2560	2560	2048	1024	2560	1280	1280	1024	1024	640	< 10	< 5	8	32
1056	160	160	128	128	160	80	80	128	128	320	< 10	< 5	8	8
1057	160	160	256	256	160	160	160	128	128	160	< 10	< 5	16	16
1058	320	160	1024	512	320	160	160	512	256	320	< 10	< 5	8	8
1059	40	40	128	128	40	40	40	32	32	320	< 10	< 5	8	16
1060	160	160	128	128	160	160	80	64	128	1280	< 10	< 5	8	8
1061	80	80	128	128	80	80	80	64	128	640	< 10	< 5	8	< 4
1062	320	320	512	256	320	320	320	256	256	640	< 10	< 5	8	8
1063	160	80	512	512	320	160	160	256	256	320	< 10	< 5	< 4	8
1064	2560	2560	2048	2048	2560	2560	2560	1024	1024	1280	< 10	5	32	64
1065	320	160	128	128	320	320	160	256	256	640	< 10	< 5	8	8
1066	320	320	512	512	320	320	320	256	256	640	< 10	< 5	< 4	8
1067	< 10	5	32	16	20	20	20	8	16	80	< 10	< 5	< 4	8
1068	2560	2560	2048	2048	2560	1280	1280	1024	1024	160	10	10	16	16
1069	20	20	32	32	80	40	40	16	32	160	< 10	< 5	< 4	8
1070	80	40	128	128	160	160	80	128	128	160	< 10	< 5	< 4	8
1071	1280	640	1024	1024	1280	1280	1280	1024	1024	640	< 10	< 5	< 4	16
1072	640	640	2048	1024	1280	1280	640	512	1024	1280	< 10	< 5	4	8
1073	320	160	1024	1024	640	320	230	512	256	320	< 10	< 5	< 4	8
1074	320	320	256	256	320	320	320	256	256	40	< 10	< 5	8	32
1075	20	20	8	32	40	20	10	8	8	1280	< 10	< 5	< 4	< 4
1076	5120	5120	8192	8192	2560	2560	2560	2048	2048	2560	40	20	512	512
1077	640	160	8192	8192	1280	1280	640	1024	1024	2560	< 10	< 5	512	1024
1078	640	320	2048	2048	1280	640	640	1024	1024	640	< 10	< 5	16	16
1079	1280	320	2048	512	1280	640	640	1024	512	640	< 10	< 5	128	16
1080	40	20	32	64	80	40	40	64	32	640	< 10	< 5	< 4	< 4
1081	320	320	1024	1024	640	320	320	512	512	640	< 10	< 5	16	32
1082	320	160	1024	512	640	640	320	256	512	320	< 10	< 5	< 4	8
1083	640	320	1024	512	640	640	640	512	512	640	10	5	16	32
1084	320	320	256	512	640	320	320	256	512	160	< 10	5	16	32
1085	640	320	1024	1024	640	640	320	256	256	1280	< 10	< 5	32	64
1086	80	40	128	128	160	80	80	64	64	640	10	< 5	8	8
1987	160	80	256	256	160	160	160	256	128	320	< 10	< 5	8	8
1088	< 10	< 5	< 4	< 4	< 5	< 10	< 5	< 4	< 4	640	< 10	< 5	< 4	< 4
1089	1280	640	1024	1024	1280	1280	640	512	512	320	< 10	< 5	16	16
1090	40	40	64	64	40	40	40	32	32	160	< 10	< 5	< 4	< 4
1091	320	160	256	512	320	160	160	256	256	80	< 10	< 5	8	< 4
1092	160	80	64	128	160	320	320	128	128	160	< 10	< 5	< 4	8
1093	2560	1280	2048	2048	1280	1280	1280	1024	1024	320	< 10	5	32	64
1094	160	80	256	256	160	320	160	128	128	640	< 10	< 5	4	4
1095	320	160	256	128	320	320	320	256	256	160	< 10	< 5	< 4	8

[1] After flotation centrifugation.

[2–6] Sera and fractions were tested after treatment with kaolin (K), Aerosil (A), dextran sulphate/$CaCl_2$ (D), heparin/$MnCl_2$ (H) or untreated (U).

https://doi.org/10.1017/S0022172400069060 Published online by Cambridge University Press

Table 3. *Sera tested by gel diffusion for residual α_1-lipoproteins after treatment.*

Serum	Reactions after treatment with: Kaolin	Aerosil	Dextran	Heparin
1049	—[1]	—	+ + (< 4)[2]	+ + (< 4)
1051	—	—	— (32)	— (64)
1059	—	+	+ + + (8)	+ (16)
1067	—	—	+ + + (< 4)	+ (8)
1068	— (10)	— (10)	+ + + (16)	+ + (16)
1070	—	—	+ (4)	+ (8)
1071	+	+	+ + + (4)	+ + (16)
1975	—	—	+ (< 4)	+ (< 4)
1076	+ (40)	+ (20)	+ + + (512)	+ + (512)
1077	(+)	(+)	+ + + (512)	+ + + (1024)
1078	+	+	+ + + (16)	+ + (16)
1079	—	—	+ + + (128)	+ (16)
1080	+	+	+ + + (< 4)	+ + (< 4)
1081	(+)	(+)	+ + + (16)	+ + (32)
1084	—	— (5)	+ + + (16)	+ (16)
1085	—	(+)	+ + + (32)	+ + (64)
1086	— (10)	— (5)	+ + (8)	+ (8)
1093	—	— (5)	+ (32)	— (64)
1094	—	—	+ (4)	+ (4)
1095	—	—	+ + + (< 4)	— (8)

[1] No precipitation line. + + + indicates precipitation line as heavy as for untreated serum; (+) indicates a faint, but visible line; + and + + indicate intermediate reactions.

[2] Figures in brackets: Rubella HI titres of top fractions after flotation centrifugation of pretreated sera.

Table 4. *Residual concentrations of immunoglobulins after treatment*

Treatment	Relative concentrations of: IgG	IgM	IgA
Kaolin	74 (68–81)[1]	72 (61–81)	83 (73–96)
Aerosil	79 (71–89)	71 (63–77)	78 (68–93)
Dextran	81 (74–89)	69 (63–74)	85 (79–98)
Heparin	76 (66–84)	74 (62–84)	81 (75–96)

[1] Results are expressed as a percentage of the value for untreated sera and represent the mean values for 48 sera (the figures in brackets give the range of values obtained).

one serum showed a positive reaction after Aerosil treatment, while 15 sera still reacted after dextran sulphate/$CaCl_2$ treatment and 12 after heparin/$MnCl_2$ treatment.

Removal of immunoglobulins. All 48 sera were tested and the results are presented in Table 4 as the percentages of immunoglobulins left after treatment. No obvious differences were found in the immunoglobulins remaining after the various methods of removal of NSIs.

Table 5. *Comparison of rubella HI titres for* 612 *sera treated in parallel with kaolin and Aerosil*

Kaolin / Aerosil	< 10	10	20	40	80	160	320	640	1280	2560	5120	10240
< 5	131	—	—	—	—	—	—	—	—	—	—	—
5	6	5	—	—	—	—	—	—	—	—	—	—
10	1	10	3	—	—	—	—	—	—	—	—	—
20	—	1	23	12	2	—	—	—	—	—	—	—
40	—	—	3	29	20	2	—	—	—	—	—	—
80	—	—	—	6	76	38	6	—	—	—	—	—
160	—	—	—	1	9	56	31	4	—	—	—	—
320	—	—	—	—	1	3	38	11	1	—	—	—
640	—	—	—	—	—	2	4	29	9	—	—	—
1280	—	—	—	—	—	—	—	3	16	5	1	—
2560	—	—	—	—	—	—	—	—	—	5	2	—
5120	—	—	—	—	—	—	—	—	—	2	4	—
10240	—	—	—	—	—	—	—	—	—	—	—	—
20480	—	—	—	—	—	—	—	—	—	—	—	1

Comparison of kaolin and Aerosil treatment in routine rubella serology

During the period from June 1978 to March 1980, 612 sera were treated with both kaolin and Aerosil, and tested in parallel for rubella HI antibodies. The correlation between the titres found in these sera is shown in Table 5. The gmt for positive sera after kaolin treatment was 154, and after Aerosil treatment 124. Seven sera which were negative (< 10) after kaolin, were positive (six sera with a titre of 5, one with a titre of 10) after Aerosil treatment. This was reproduced five times.

Identical titres were found in 418 sera (68%). In 136 sera (22%) the titres were 1 dilution step higher after kaolin and in 31 (5%) after Aerosil treatment. Significant titre differences (2 dilution steps) were seen in 20 sera (3·3%).

For all patients with a significant, diagnostic HI titre increase ($\geq$ two dilution steps) in two consecutive serum samples, the diagnosis was made after both pretreatments.

The specificity of rubella HI titres less than 10 *after Aerosil treatment*

During our studies we have detected 10 sera which are negative (no HI activity at a 1 in 10 dilution) after kaolin, and positive (HI titre 2–8) after Aerosil treatment. These sera have been treated at least twice, and titrated repeatedly. Sufficient remained of only 8 of these sera for further testing.

These eight sera were examined by HI after flotation centrifugation and by HIG. Six of the sera seemed to contain rubella antibodies. Rubella HI activity was found in the bottom fractions of these sera, and they gave HIG reactions corresponding to the HI titre. Two sera had HI activities in the top fractions and gave no HIG haemolytic zones. These sera were regarded as false positives due to inhibitors of unknown nature. The conclusion was supported by concentration and retesting by HIG. The haemolysis diameters increased as expected for the six positive sera, while no haemolysis was seen for the two false positives.

DISCUSSION

The ideal procedure for exclusion of non-specific inhibitors of rubella HA should remove lipoproteins completely with a minimum loss of immunoglobulins. It should allow detection of low antibody titres and be rapid and simple to perform at a reasonable price. None of the methods evaluated in these studies are satisfying such demands.

The low density lipoprotein (LDL) class seems to include the most potent inhibitors (Chang & Weinstein, 1972; Blom & Haukenes, 1974; Shortridge & Ho, 1974; Campbell, 1977; Ho & Shortridge, 1977 and Steinmann, 1977*a*, *b*). But activity may also be found in the high density lipoprotein (HDL) and very low density lipoprotein (VLDL) fractions of sera (Blom & Haukenes, 1974; Shortridge & Ho, 1974; Haukenes & Blom, 1975 and Shortridge & Ho, 1976). The total concentrations and inhibitory activities of different lipoproteins may be subject to individual variations and also be influenced by various physiological and pathological conditions (Beaumont *et al.*, 1970; Haukenes & Blom, 1975 and Ellis & Campbell, 1977). All serum lipoproteins should therefore be reliably excluded before rubella HI testing.

The polyvalent anion-divalent cation combinations used in these studies, heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$, left non-specific inhibitors in a majority of the sera, and in considerable amounts in some of them. In fact, rubella HI titres below 32 may just as well be due to non-specific inhibitors as to specific antibodies. This relative failure was confirmed by the findings of residual HDL in a majority of sera tested after treatment with heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$. The last mentioned method was least favourable in this respect. Other studies (Haukenes & Blom, 1975; Ellis & Campbell, 1977 and Steinmann, 1977*a*, *b*) have shown that heparin/$MnCl_2$ fail to remove HDL, VLDL and occasionally also LDL. Dextran sulphate has been claimed more structurally fit for lipoprotein exclusion (Bernfeld *et al.* 1960). But dextran sulphate of different molecular weights and from different manufacturers has shown variable effectiveness (Nelson, Quiring & Inhorn, 1972; Haukenes & Blom, 1975 and Ellis & Campbell, 1977).

Sera which contained HDL after treatment did not always have residual rubella HI activity and vice versa, indicating that HDL do not always include non-specific inhibitors.

Both kaolin and Aerosil treatment left less non-specific inhibitors and HDL than the two polyvalent anion-divalent cation combinations. Aerosil treatment was the most effective procedure. The results suggested that low rubella HI titres could be looked upon with some confidence after kaolin and Aerosil treatment. Earlier studies (Schmidt & Lennette, 1970 and Haukenes & Blom, 1975) have shown that kaolin occasionally may leave non-specific inhibitors. But comparative studies indicated that kaolin treatment was more reliable than polyvalent anion-divalent cation combinations (Haukenes & Blom, 1975 and Steinmann, 1977*a*).

The four methods investigated gave similar results with regard to removal of

immunoglobulins. These findings are consistent with those of others (Feldman, 1968; Schneweis, Wolff & Marklein, 1972; Haukenes & Blom, 1975 and Ellis & Campbell, 1977). The two available methods which do not remove immunoglobulins, flotation centrifugation (Blom & Haukenes, 1974) and phospholipase C treatment (Haukenes & Blom, 1975 and Iwasa & Hori, 1976) are laborious and expensive.

Removal of specific rubella antibodies by pretreatment was indicated by the HI titres in flotation centrifugation fractions before and after treatment. The gmt of the bottom (immunoglobulin) fractions after kaolin treatment was somewhat higher than after the other methods. Blom & Haukenes (1974) have published similar findings. They suggested that kaolin removed some blocking factor, and that this could compensate for loss of immunoglobulin.

It may be important to detect low rubella HI titres for diagnostic and sero-epidemiological purposes, and also in immunosurveillance and vaccination control. Technical problems (Biano, Chang & Daniels, 1974; Allen & Hedlund, 1976 and Ellis & Campbell, 1977) with reading HI titres of four after treatment with heparin/$MnCl_2$ and dextran sulphate/$CaCl_2$ can be avoided. But the residual non-specific inhibitors and lipoproteins mean that low HI titres cannot be relied upon.

More confidence can be placed upon HI titres < 10 after Aerosil treatment. However, 2 out of 8 sera with such titres were shown to be false positives. Very low titres should therefore be confirmed by flotation centrifugation and/or HIG.

In conclusion, among the four methods for exclusion of non-specific inhibitors evaluated, Aerosil and kaolin treatment are the methods of choice. For laboratories already employing the kaolin method, there are few advantages connected with a conversion to Aerosil treatment. However, the latter method offers an opportunity to detect low rubella HI titres.

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