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Validation of a Volunteer Model of Cholera with Frozen Bacteria as the Challenge

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To evaluate a standardized inoculum of *Vibrio cholerae* for volunteer challenge studies, 40 healthy adult volunteers were challenged at three different institutions with a standard inoculum prepared directly from vials of frozen, virulent, El Tor Inaba *V. cholerae* N16961, with no further incubation. Groups of 5 volunteers, with each group including 2 volunteers with blood group O, were given a dose of 10^5 CFU, and 34 of the 40 volunteers developed diarrhea (mean incubation time, 28 h). Transient fevers occurred in 15 (37.5%) of the volunteers. *V. cholerae* was excreted by 36 of 40 volunteers. Five additional volunteers received 10^4 CFU, and four developed diarrhea but with a lower average purging rate than required for the model. Of the 40 volunteers, 37 developed rises in their vibriocidal and antitoxin titers similar to those in previous groups challenged with freshly harvested bacteria. We conclude that challenge with frozen bacteria results in a reproducible illness similar to that induced by freshly harvested bacteria. Use of this model should minimize differences in attack rates or severity when groups are challenged at different times and in different institutions.

Cholera continues to be a major public health problem in nearly all developing countries, now including the Western hemisphere (17). It became newsworthy recently because of its introduction into Latin America in 1991 and because of the epidemic among refugees in Zaire, which led to an estimated 50,000 deaths during this epidemic alone (7). Cholera continues in less dramatic fashion as an endemic disease in over 100 countries (6). An improved vaccine(s) for cholera could provide an important public health tool with which to control the disease (4). The development of such a vaccine has been aided greatly by the use of volunteer studies in which volunteers are immunized with an experimental vaccine and then experimentally challenged with a virulent strain while hospitalized in a facility experienced in the management of such volunteers. By comparing the rates and severity of illness in immunized and nonimmunized volunteers, the efficacy of the vaccine against this standard challenge is determined (2, 9, 10, 14–16). Experience with this model has indicated that vaccines showing efficacy in volunteers have also demonstrated efficacy when tested in field trials (2).

While the volunteer model is valid and useful, there are constraints which have limited its utility. The major constraint relates to the need for each group of volunteers to receive the same virulent challenge, in terms of both the numbers of bacteria and the virulence of the bacterial preparation. Currently, the bacterial challenge is prepared by a standard operating procedure so that a consistent number of freshly grown bacteria is given to the volunteers. However, only a few vaccine-testing centers have the resources to develop this challenge procedure. In addition, minor differences in growth conditions (e.g., media, time of incubation, and temperature) could affect the virulence of the challenge bacteria. As the need grows for additional testing sites where such volunteer studies can be performed, there is also a need to ensure that the challenge given to volunteers is uniform among different volunteer units and among groups of volunteers within the same unit challenged at different times.

The National Institutes of Health therefore undertook to prepare a batch of frozen *Vibrio cholerae* (El Tor Inaba strain N16961) with a large number of aliquots so that identical vials from the lot could be used for volunteer challenge studies. It was hypothesized that the use of this frozen inoculum would result in consistent attack rates, and consistent severity of illnesses among different groups of volunteers and at different clinical sites. Furthermore, it was hypothesized that the illness seen when frozen inocula were used would be similar to illnesses observed when freshly harvested bacteria were used, including a geometric mean stool output of $\geq$3 liters.

Since the blood group of the volunteer is also an important determinant of the severity of cholera (1, 3, 8), the model also included two persons with blood group O among each group of five volunteers to standardize this potentially confounding variable.

This report summarizes the clinical, microbiological, and serological findings when the frozen inoculum was given to nine groups of volunteers at three institutions. Past studies have used a dose of 10^6 CFU per dose of freshly harvested organisms; hence, the plan was to begin with a lower dose (10^5 CFU), expecting to increase in 1-log increments up to 10^6 or even 10^7 CFU per dose if needed to induce a consistent illness.

MATERIALS AND METHODS

Preparation and validation of the inoculum. The challenge lot, containing El Tor strain N16961, was prepared as a 5-liter fermentor batch, and over 2,300 vials with 2 ml per vial were filled, labeled, and frozen at −70°C by the Salk Institute, West Point, Pa. This strain has been used frequently as a challenge strain by the University of Maryland Center for Vaccine Development (CVD) (10). Sample vials were tested to validate the purity and stability of the inoculum. The frozen

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TABLE 1. Clinical and bacteriological responses in groups of volunteers challenged with 10^5 *V. cholerae* El Tor N16961 frozen inoculum preparation

Group[a]	No. with diarrhea	No. with >3,000 g of diarrhea	Mean vol of diarrhea (g)	Mean incubation time (h)	No. with vomiting	No. with fever >100.5°F (maximum fever [°F])	No. with positive stool cultures	GMT of peak quantitative cultures
JHU 1	5	3	2,640	28	1	3 (102.2)	5	1.4×10^7
JHU 2	4	1	2,658	21	1	0	5	1.5×10^6
JHU 3	4	3	4,623	27	2	3 (101.0)	4	1.4×10^8
UM 4	4	3	5,476	21	1	2 (103.5)	5	1.4×10^8
UM 5	4	1	2,827	32	1	2 (102.5)	4	1.0×10^8
UM 6	3	2	3,864	34	1	3 (101.8)	3	1.6×10^8
CHC 7	5	2	2,794	29	3	1 (100.8)	5	8.1×10^7
CHC 8	5	3	3,844	35	0	1 (101.6)	5	3.0×10^7
Total	34	18	3,416	28	10	15 (103.5)	36 (90%)	3.9×10^7

[a] There were five patients per group.

preparation was agglutinated with *Vibrio* O1 ("poly") antiserum (Difco Laboratories) and with Inaba antiserum but not with Ogawa antiserum or saline. These results were identical to those exhibited by the positive control strain obtained from the culture collection at the CVD. Both the frozen and control samples "keyed" to *V. cholerae* when the 20E strip (API Analytabs, Plainview, N.Y.) was used. A total of 90 colonies from three frozen vials were obtained from Luria agar plates and were hybridized with a probe to detect cholera toxin-encoding genes (10). All 90 colonies hybridized with the cholera toxin probe. The concentration of organisms (CFU/per milliliter on Luria agar) has been consistently 5×10^9 since the preparation of the challenge lot in August 1995 until the present (February 1997). The vials are stored at a National Institute of Allergy and Infectious Diseases repository and were shipped on dry ice to the participating study sites. The vials were kept frozen at −70°C until used at the respective sites, and the contents were diluted in phosphate-buffered saline immediately before use. The stability of the inocula was further documented by obtaining quantitative counts with serial dilutions in the laboratory of the volunteer unit. After incubation of the inoculum in 1.33% sodium bicarbonate buffer for 1 h, there was no change in the numbers of bacteria recovered.

Study plan. The goal of the study was to determine a dose of bacteria which would consistently induce diarrhea in ≥80% of volunteers. Additionally, it was important that many of the illnesses should be moderate or severe (i.e., that the geometric mean of the total diarrhea was at least 3 liters). For safety reasons, it was planned to start with a dose of 10^5 bacteria (1 log lower than has been used with freshly grown bacteria) and to increase the inoculum strength if the attack rate or the severity was too low. Since the dose of 10^5 did, in fact, induce illnesses according to the criteria established, the dose was not increased, but one group was given a lower dose (10^4 CFU) to determine if an even lower dose would be sufficient to be useful in the model.

Informed consent was obtained from all subjects, and experimentation followed the guidelines of the U.S. Department of Health and Human Services and of the respective institutions (Johns Hopkins University, the University of Maryland, and Children's Hospital Medical Center).

Volunteer studies. Groups of volunteers ($n = 5$ per group) were recruited from the community in Baltimore and Cincinnati for our studies by advertisements in local newspapers. Healthy, eligible, and willing volunteers between 18 and 40 years of age were trained, passed a written examination to document their knowledge of cholera and the procedures of the study, signed an informed-consent form, and underwent a series of clinical and laboratory examinations to rule out occult illness or pregnancy. The screening examinations included a complete blood count with differential, chemistry panel, urinalysis, hepatitis B antigen, hepatitis C antibody, human immunodeficiency virus antibody, blood type, and urine human chorionic gonadotropin (for females) within 2 days of the study, and an electrocardiogram. Potential volunteers were excluded if any of the following applied: chronic illness, immunosuppressive condition, human immunodeficiency virus antibody positive, hepatitis B surface antigen positive, hepatitis C antibody positive, travel to an area of endemic cholera infection within 5 years, receipt of a cholera vaccine within 5 years, previous participation in a research study with cholera or enterotoxigenic *Escherichia coli*, pregnancy (urine human chorionic gonadotropin), antibiotic administration within 7 days of the challenge, regular use of laxatives, abnormal stool pattern, significant abnormality in screening laboratory hematology and chemistry tests, known allergy to tetracycline, or a significant abnormality on EKG. Within each group of five volunteers, two had blood group O.

Volunteers were admitted to the study unit 1 or 2 days before challenge. On the day of challenge (day 0), they ate breakfast at about 7 a.m., and then fasted until the challenge, which occurred at about 10 a.m. The challenge inoculum was made by thawing a single vial of frozen preparation and serially diluting it in phosphate-buffered saline by a standard operating procedure. The inocula were placed into separate plastic bottles containing 30 ml of the bicarbonate solution. During the challenge procedure, the volunteers drank 120 ml of buffer solution and 1 min later drank the bacterial challenge dissolved in the same buffer solution. The total amount of buffer was 2 g of sodium bicarbonate in 150 ml of water. After the challenge, they fasted for 90 min and then were allowed to eat food ad libitum.

Clinical management. The volunteers were monitored for the occurrence of symptoms such as nausea, vomiting, and diarrhea and were visited at least twice daily to ascertain and manage any medical problems. Fluid intake and output was continuously monitored, with subtotals being obtained every 8 h. All stools were passed into a plastic container for weighing, inspecting, sampling, and disinfection (with sodium hypochlorite [Clorox]). The stools were graded on a 1 to 5 scale (1, firm; 2, soft; 3, runny, takes the shape of the container; 4, brown liquid; 5, rice water). The hydration of volunteers who developed diarrhea was maintained with either a glucose- or rice-based oral rehydration solution (CeraLyte, Jessup, Maryland) to match diarrhea output losses with oral rehydration solution on a 1.5:1.0 basis (one-and-a-half times as much oral rehydration solution as diarrheal stool output). When clinically indicated, intravenous Ringer's lactate was given, matching diarrhea stool volumes with an equivalent volume of rehydration fluid.

Tetracycline administration (500 mg orally every 6 h for 5 days) was started at the time the volunteers met the definition of severe cholera (>5 kg of diarrhea stool) or on day 4 at 12 noon if they had not already received it. They were discharged when they were asymptomatic, had negative stool cultures for *V. cholerae* on two samples, and had received a complete course of tetracycline.

Definitions. A patient with cholera was defined as a volunteer who passed at least two diarrheal stools (grade 3, 4, or 5) totaling at least 200 g or who passed at least one diarrheal stool totaling 300 g within a 48-h period, associated with a stool culture positive for *V. cholerae*. A patient with a moderate case was one who passed 3 to 5 kg of diarrheal stool during the study, and one with a severe case was one who passed at least 5 kg during the study.

Laboratory procedures. The clinical screening laboratory tests were carried out in the clinical laboratories of the participating hospitals. The microbiology tests were carried out in the research laboratories of the respective investigators.

Stool cultures were obtained daily (up to two specimens per day) after the challenge to determine the duration of excretion of virulent *V. cholerae* and to measure the number of *V. cholerae* being excreted. A rectal swab specimen was obtained if no stool was passed. The fecal specimens were inoculated into alkaline peptone water for qualitative culture and diluted 1:10 in PBS for quantitative culture. The alkaline peptone water specimen was inoculated onto a thiosulfate-citrate-bile salts-sucrose (TCBS) plate after a 6-h incubation at 37°C. For quantitative cultures, 100 μl of stool diluted serially (10-fold dilutions) was spread on TCBS agar and incubated at 35°C and the numbers of colonies of *V. cholerae* were counted after an 18-h incubation. Suspected colonies were confirmed to be *V. cholerae* on the basis of results with oxidase reagent and agglutination with O1 antiserum.

Blood samples (20 ml for serological testing and 35 ml for antibody-secreting cells assays) were obtained four times: before admission and on days 7, 10, and 14. Aliquots of serum were stored at −20°C until assayed for cholera serology. To compare the serological results at two laboratories, aliquots from the first three groups from both the Johns Hopkins University Vaccine Testing Unit (JHU-VTU) and the CVD (120 serum samples) were sent to the National Institutes of Health, where the sera were coded and sent back to the laboratories of the JHU-VTU and the CVD, where they were tested for vibriocidal and antitoxin antibodies. These coded sera were arranged so that the four specimens from each volunteer were tested in adjacent wells, but the order of the sera was randomized within each group of four samples.

Methods used at these laboratories have been described previously (5, 11, 13). The methods used for vibriocidal antibody assays were similar between the two institutions, and for both the end point was defined as the highest dilution in which there is no visible growth. The antitoxin assay, however, is performed differently; the JHU-VTU laboratory performs the assay with serial fivefold

TABLE 2. Comparison of the clinical and bacteriological responses in volunteers challenged with *V. cholerae* El Tor N16961 frozen inoculum preparation by blood group

Blood group	No. with diarrhea/total no. (%)	No. with >3,000 g of diarrhea/total no. (%)	No. with >5,000 g of diarrhea/total no. (%)	Mean diarrhea vol (g)	Mean incubation time (h)	No. with vomiting/total no. (%)	No. with fever >100.5°F/total no. (%)	No. with positive stool cultures/total no. (%)	GMT of quantitative cultures
O	15/16 (94%)	9/16 (56%)	7/16 (44%)	4,068	27	3/16 (19%)	6/16 (38%)	15/16 (94%)	3.6×10^7
Non-O	19/24 (79%)	9/24 (38%)	3/24 (13%)	2,977	30	7/24 (29%)	9/24 (38%)	21/24 (88%)	4.2×10^7

dilutions of sera to reach an end point (defined as the extrapolated titer yielding an optical density [OD] of 0.4), while the CVD laboratory determines the OD with a single 1:50 dilution of serum. A positive response is defined as an increase of 0.2 OD unit. Thus, the JHU-VTU laboratory result is an end point titer whereas the CVD result is read as positive or negative.

At the same time as the serum collections, whole blood was also collected to determine the number of antibody-secreting cells (ASC) to cholera toxin and *V. cholerae* lipopolysaccharide (LPS). This assay was carried out at the CVD (12). The results were expressed as the number of specific antibody-secreting cells/10^6 peripheral blood mononuclear cells.

Comparison to past challenge studies. Records from past studies conducted at the CVD with freshly harvested *V. cholerae* N16961 were reviewed to compare the illnesses which occurred in the past studies with those seen in the present study, which used the same strain but with a frozen inoculum.

RESULTS

Challenge dose of 10^5 CFU. Eight groups of volunteers (n = 5 per group) received a challenge dose of 10^5 CFU, including three groups at JHU-VTU, three at CVD, and two at Children's Hospital Medical Center, Cincinnati.

The clinical illnesses observed among all groups of volunteers were, in general, typical of cholera, as shown in Tables 1 and 2. Of the 40 volunteers, 34 (85%) developed diarrhea; the attack rates in the groups varied from 60 to 100%. Only one group among the eight had fewer than four illnesses. Of these illnesses, 16 (47%) were mild, 8 (24%) were moderate, and 10 (29%) were severe. As expected, the most prominent symptom of the illnesses was watery diarrhea, typical of cholera. Ten (25%) of the volunteers also vomited. The interval between ingesting the inoculum and the onset of symptoms averaged 28.4 h (standard deviation SD = 8.7 h; range = 11 to 48 h), and most symptoms started the day after challenge.

As expected, volunteers with blood group O tended to have more severe diarrhea than others, although this was of borderline statistical significance (Table 2). Of 16 volunteers with blood group O, 7 had severe diarrhea, but only 3 of 24 with other blood groups had severe diarrhea (P = 0.058, Fisher's exact test). If those having either moderate or severe diarrhea are grouped, however, there was no difference by blood group (9 of 16 for blood group O versus 9 of 24 for other groups). The geometric means of the diarrheal stool volumes were 4,068 g (confidence interval = 2,541 to 6,512 g) for blood group O and 2,977 g (C.I. = 2,019 to 4,389 g) for other groups (not statistically significant).

Fever occurred more often than expected. Fevers (>100.5°F) occurred the day after challenge and lasted less than 24 h in 15 (37.5%) of the 40 volunteers. The fevers averaged 101.4°F, but one volunteer's temperature reached 103.5°F. The volunteers with fever complained of malaise, but there were no localizing complaints associated with the fever. Blood cultures from the first three febrile volunteers were sterile.

Stool cultures from all but four volunteers who were challenged were positive (Table 1). None of these four had diarrhea. Two other asymptomatic volunteers did have positive stool cultures. All persons with diarrhea had a positive stool culture for *V. cholerae*, and the concentration of *V. cholerae* was generally from 10^6 to 10^9 CFU per g of feces (geometric mean, 3.9×10^7 CFU/g).

Challenge dose of 10^4 CFU. At JHU-VTU, one group (n = 5) was challenged with 10^4 CFU to determine if this lower dose would be adequate. Four of five volunteers did develop diarrhea; however, the geometric mean stool output was 1,608 g, which is less than required for the model. Three of these volunteers also had transient fever (maximum temperature = 101.7°F), including one who did not have diarrhea. Stool cultures from each volunteer were positive.

Serological test results. A total of 37 (92.5%) of 40 volunteers who received 10^5 CFU and 5 of 5 who received 10^4 CFU had significant rises in their vibriocidal and antitoxin titers when prechallenge sera were compared with sera collected on days 10 and 14 after challenge. The results of the serum antibody assays on samples from the first 30 volunteers which were assayed at both the JHU-VTU and the CVD under code are shown in Table 3. The volunteers included five groups challenged with 10^5 CFU (n = 25) and one group challenged with 10^4 CFU (n = 5). Of 40 volunteers, 37 (92.5%) developed a ≥fourfold vibriocidal response and a ≥twofold immunoglobulin G (IgG) (and IgA at the JHU-VTU) antitoxin response when the day 10 and day 14 sera were compared to the prechallenge sera. The three volunteers who failed to develop serological responses were ones who also had no diarrheal symptoms, but three other asymptomatic volunteers did de-

TABLE 3. Serological responses among 30 volunteers challenged with frozen *V. cholerae* N16961 as measured in two laboratories

Characteristic	Value obtained at[a]:	
	Johns Hopkins University	University of Maryland
Vibriocidal titer (no. with ≥4-fold rises)	27	27
Before challenge	5.9 (5.01–6.9)	24.6 (16–38)
Day 7	65 (37–114)	313 (183–535)
Day 10	285 (146–553)	1,437 (722–2,856)
Day 14	266 (137–514)	1,280 (638–2,567)
GMT of the rise (day 10)	48.5 (25–93)	58 (24–139)
Antitoxin IgG (no. with significant rises)	27	27
Before challenge	10.9 (8.9–13.4)	NA[b]
Day 7	66.0 (38.0–114)	NA
Day 10	305 (144–649)	NA
Day 14	630 (318–1,249)	NA
Antitoxin IgA (no. with significant rises)	27	
Before challenge	28.1 (19.4–40.7)	NA
Day 7	150 (82.1–277)	NA
Day 10	515 (251–1,058)	NA
Day 14	764 (407–1,432)	NA

[a] GMT are shown (upper and lower bounds of the 95% confidence intervals).
[b] NA, not applicable.

TABLE 4. ASC responses in volunteers challenged with frozen N16961 *V. cholerae*

Time (day)	No. of volunteers	Mean no. of ASCs per ml to:					
		Anti-Inaba LPS			Antitoxin		
		IgA	IgG	IgM	IgA	IgG	IgM
0	26	<1	0	<1	<1	0	<1
7	30	18	1	44	121	83	9.6
10	27	27	4.4	58	77	72	8.5

velop vibriocidal and antitoxin responses. All symptomatic volunteers developed significant vibriocidal and IgG (and IgA at the JHU-VTU) antitoxin responses. Peak vibriocidal titers were seen on day 10, while peak antitoxin titers were seen on day 14 (Table 3).

When blinded samples were used, there was 100% agreement between the two laboratories with regard to which individuals developed significant vibriocidal and IgG antitoxin responses. The geometric mean titers (GMTs) of the vibriocidal titers tended to be about four to five times higher at the CVD than at the JHU-VTU. The correlation coefficient between the logs of the absolute titers was 0.84. Despite some differences in the titers of individual sera, the magnitude of the rises in the geometric mean titers was similar between the two laboratories (about a 50-fold increase between prechallenge and day 10 sera) and the correlation between the log of the rises was 0.75. Differences in methods for IgG antitoxin responses preclude direct comparison of the magnitude of the IgG antitoxin responses. All persons who developed a rise in IgG antitoxin titer also developed a significant rise in IgA antitoxin titer as determined at the JHU-VTU laboratory.

Immune responses, as determined by increases in the numbers of ASCs in peripheral blood occurred in most of the volunteers. The mean numbers of ASCs to cholera toxin and Inaba LPS are shown in Table 4. IgA and IgG ASC antitoxin responses predominated, with the responses being somewhat higher on day 7 than day 10. The same three volunteers who had no vibriocidal or antitoxin response also failed to develop a detectable IgA or IgG antitoxin ASC response and remained asymptomatic. Anti-Inaba LPS ASC responses were less frequently detected than were antitoxin responses. With an arbitrary cutoff of 5 ASCs per 10^6 peripheral blood mononuclear cells 15 volunteers developed rises in anti-LPS IgA ASCs and only 4 developed IgG ASC responses. By contrast, 26 of 29 developed IgM anti-LPS ASC responses.

DISCUSSION

This study, involving eight similar groups from three vaccine-testing centers, has demonstrated that ingestion of an inoculum prepared directly from a frozen vial of *V. cholerae* N16961, with no further incubation, resulted in a consistent, acute, watery diarrheal illness typical of cholera. The incubation period and time course were similar to those in previous studies with freshly harvested N16961. A comparison of the results obtained in the present study (with frozen inoculum) with historical data from previous challenge studies performed at the CVD (with freshly harvested organisms) is shown in Table 5.

The only unusual symptom with the model used in the present study was the self-limited fever beginning the day after challenge in more than 50% of the volunteers. Fevers have been seen in volunteers challenged with this strain previously, but the proportion of volunteers with fever in these groups was higher than observed previously. The cause of the fever is not known; however, it seems most likely that the fever is related directly to infection with the *Vibrio* strain itself rather than to an adventitious agent, since the inoculum was screened carefully for contaminants. A pyrogenic contaminant or fever due to endotoxin from killed *Vibrio* organisms is most unlikely since the inoculum itself was diluted by a factor of 50,000 before being given to the volunteers. Fevers also occurred in the group receiving a dose of 10^4 CFU (dilution factor of 500,000), suggesting that the causative agent of the fever is a viable organism.

Since the challenge organism started from a frozen state, it seemed possible that either the incubation period or the immune response would be delayed relative to those seen for challenge with freshly harvested organisms. In fact, there was no difference in the time course of either of these events. Technically, the use of the frozen challenge is easier and results in a consistent inoculum. Compared to a 2-days preparation when fresh organisms are used, the frozen inoculum can now be prepared, by a standard procedure, in about 30 min. More importantly, the inoculum given to volunteers is uniform and so the identical inoculum can be given at different times and at different centers.

Since the vibriocidal and antitoxin assays are key indicators of the immune response, it seemed important to compare the results in at least two laboratories. The titers, as reflected by the GMTs were approximately four to five times higher when measured at CVD than when measured at JHU-VTU, but there was good correlation between the rises in titer between the two laboratories and there was 100% agreement in determining which volunteers had a significant vibriocidal response. The IgG responses to antitoxin also agreed between the two laboratories, but the different methods used prohibited a comparison of the magnitude of the responses.

While this study has demonstrated that challenge with a frozen inoculum results in a consistent cholera illness, further studies are needed to investigate whether immunized volunteers will be protected against this standard challenge. If these studies are successful, this will greatly assist in the efficient evaluation of candidate cholera vaccines in the future. The results of the present study may also be relevant for other volunteer studies involving oral challenge with live bacteria, e.g., *E. coli* or shigellae.

TABLE 5. Clinical illnesses observed in past challenge studies of naive volunteers with freshly harvested *V. cholerae* N16961

Study	No. of volunteers	Attack rate (%)	Mean incubation period (h)	Mean diarrhea stool vol (g)	No. with fever of >101°F	% with O blood group
3002	5	100	23	4,530	1	60
3005	5	80	25	1,606	0	60
5009	8	75	28	4,923	2	50
5011	7	71	26	3,931	1	29
5013	8	88	22	4,543	1	63
6001	7	86	32	2,520	0	29
6005	11	91	26	4,024	0	55
6011	6	100	31	9,415	1	83
7002	7	100	32	3,473	0	67
7004	8	75	26	2,147	0	25
9003	8	88	19	2,902	1	25
11002	13	100	21	2,603	3	38
Total	93	88	26	3,885	10	47

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