Monotest in the complement fixation test: the Chorus system

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SUMMARY
The complement fixation test (CFT) is a method used for the detection of antibodies against pathogens of infectious diseases, it has been proved to be a useful diagnostic method in the detection of acute disease in many laboratories. The medical tests performed manually is time consuming and needs very skilled personnel. This study evaluates the automated Chorus CFT system with 87 serum samples in comparison with manual method using Virion-Serion reagents, against a panel of antigens, such as Adenovirus, Influenza A and B viruses, Respiratory Syncythial Virus, Parainfluenza Mix, Mycoplasma Pneumoniae, and Echinococcus. The Chorus system includes standardized reagents and a monotest device to perform the single assay. In comparison to the manual CFT method, the correlation is 91.6% (7/83). The results obtained show that the automated Chorus system can be applied for detecting complement fixation antibodies against different infectious disease agents.

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INTRODUCTION
The complement fixation test (CFT) is an immunological serological method for the detection of antibodies against pathogens responsible for viral, bacterial and parasitic infectious diseases (3). Despite the existence of advanced techniques and methods, the CFT test is a diagnostic method still used in the labs, particularly to detect some viruses responsible for respiratory diseases, such as Adenovirus, Influenza A and B virus, Parainfluenza and, although with some limitations, respiratory syncythial virus (4, 5, 6, 7).
The CFT test is specially indicated for the determination of the acute phase of an infectious disease, and the assessment of the levels of re-infection or reactivation, without any distinction among classes of antibodies, IgG and IgM antibodies.
In the case of respiratory viruses, the immune response to frequent re-infection, is quite strong and often sudden, and it is more intense than the original response to the primary infection. The detection of the specific antibody with the CFT test is a useful alternative, especially in case of diagnosis of acute respiratory infections, compared with the methods based on the direct detection of the antigen.

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The isolation of the infectious agent from clinical samples is not always easy to perform, and the tests that directly detect the antigens do not provide the required specificity. The molecular analysis for the detection of the genome of most bacterial and viral agents, although very helpful, is not well standardized for use in clinical practice. The use of CFT, as screening test of samples for many different antigens within a single session, may overcome all these limits. A CFT test is rather laborious and difficult to standardize, as also shown in the guidelines (1).
The long lead time, the training of highly qualified personnel, the results being generally interpreted subjectively, created the opportunity to develop automated systems (2). The Chorus system, versatile, designed to meet the needs of small-medium laboratories**,** has joined the already existing Seramat. The Chorus system has, compared to the Seramat, also the advantage of Monotest: a single device to perform the single assay.
This paperwork describes the evaluation of the results obtained with the Chorus system with the ones obtained with the manual method using Virion-Serion reagents against a panel of human sera.

MATERIALS AND METHODS

The study was performed on 87 human sera from 3 different laboratories and kept at -20°C, at the moment of analysis the defrosted samples were inactivated at 56°C for 30 minutes in water bath. Antibodies were determined against antigens: Adenovirus (15 sera), Influenza A virus (14 sera), Influenza B virus (12 sera), Respiratory Syncythial virus (15 sera), Parainfluenza mix (11 sera), *Mycoplasma pneumoniae* (10 sera), *Echinococcus* (10 sera).
Complement-fixing antibodies were determined in all samples using the manual method with the related Virion-Serion reagents (in the case of the *Echinococcus* the Diesse antigen was used with Virion-Serion haemolytic system), and with the Diesse Chorus system.

Manual titration method

The manual complement fixation test method has been prepared following the Kolmer standard method (overnight fixation at 2/8°C) using Virion-Serion (Würzburg, Germany) reagents. The serial dilution of serum was performed in microtitration plates after inactivation at 56°C for 30 minutes, to prevent the interference of the reaction due to the complement contained in the serum. Afterwards, antigen and complement were added in constant quantities. After overnight incubation the haemolytic system was added.
In presence of specific antibodies, the immune complexes will bind the complement preventing haemolysis of the red blood cells .
In absence of the specific antibodies against the antigen, the complement will haemolyse of the red blood cells.
The antibody titre is the highest dilution in which 50% of haemolysis occurs ; it is usually evaluated with the naked eye, observing the size of the "button" of red cells settled on the bottom of the plate.

Chorus system titration

The automated method (Chorus system; Diesse, Monteriggioni, Italy) was used to quantify antibodies against the above listed antigens in all samples included in the study. The Chorus system includes an automated processor and the diagnostic kits , the monotest device containing all reagents needed for one single test: liquid or freeze-dried antigen, freeze-dried complement and haemolysin, stabilized red blood cells. The instrument prepares all the reagents needed for analysis; it is possible to process up to 30 samples simultaneously, even with a different test for each one.
In the analytical cycle, 3 kinetic readings are performed on the 2 parallel reactions:
1) antigen and serum in presence of haemolytic system (complement, haemolysin, red blood cells)

2) serum without the addition of the antigen , in presence of the haemolytic system (complement, haemolysin, red blood cells) for the assessment of the anticomplementary power of serum. The comparison of the two kinetics is expressed in seconds, and this period of time is proportional to the serum titre, Table 1.

Table 1. *Conversion time (sec.) / titre*

0 -41 sec	NEG
41-100 sec	1/8
101-300 sec	1/16
301-500 sec	1/32
501-700sec	1/64
701 sec	1/128

Study of performances

The final titre plus or minus 1 dilution found on both methods on the same sample was considered concordant **..**

The inter-runs precision study was carried out by testing the control sera of Adenovirus, Influenza A virus, Influenza B virus and Respiratory Syncythial virus in duplicate, twice a day for 3 consecutive days. The average, the standard deviation and its CV% were calculated on a period of time expressed in seconds.

RESULTS

Evaluation of adenovirus

Table 2.

SAMPLE ID	CHORUS	VIRION
1 **adv**	neg	neg
2 adv	1/32	1/64
3 adv	1/32	1/64
4 adv	1/8	1/128 D
5 adv	A/C	1/64
6 adv	A/C	1/64
7 adv	>128	1/32 D
8 adv	1/8	1/16
9 adv	1/64	1/32
10 adv	1/16	1/32
11 adv	1/16	1/128 D
12 adv	neg	neg
13 adv	1/8	neg
14 adv	A/C	1/16
15 adv	1/16	1/32

On 15 sera, 12 were concordant according to both methods and 3 were discordant (D)

The samples: 5 adv, 6 adv, 14 showed anticomplementary power (A/C), so they were omitted from the evaluation.

Evaluation of Influenza A Virus

Table 3.

SAMPLE No.	CHORUS	VIRION
1 inf a	1/32	1/64
2 inf a	1/16	1/8
3 inf a	1/32	1/64
4 inf a	1/32	1/64
5 inf a	1/128	1/64
6 inf a	1/128	1/128
7 inf a	1/128	1/256
8 inf a	> 1/128	1/128
9 inf a	> 1/128	1/128
10 inf a	> 1/128	> 1/256
11 inf a	> 1/128	1/128
12 inf a	> 1/128	> 1/256
13 inf a	> 1/128	> 1/256
14 inf a	1/64	1/64

14 tested sera were all concordant according to both methods

Evaluation of Influenza B Virus

Table 4.

SAMPLE No.	CHORUS	VIRION
1 inf b	1/32	1/64
2 inf b	1/16	1/32
3 inf b	1/64	1/64
4 inf b	1/64	1/64
5 inf b	1/32	1/128 D
6 inf b	1/32	1/64
7 inf b	1/32	1/64
8 inf b	> 1/128	1/128
9 inf b	1/32	1/32
10 inf b	1/64	1/128
11 inf b	1/128	1/64
12 inf b	> 1/128	1/64

On 12 sera, 11 were concordant according to both methods and 1 was discordant (D)

Evaluation of Respiratory Syncythial Virus

Table 5.

SAMPLE No.	CHORUS	VIRION
1 rsv	1/64	1/16 D
2 rsv	1/16	1/8
3 rsv	1/32	1/16
4 rsv	1/64	1/16 D
5 rsv	1/16	1/8
6 rsv	1/16	1/8
7 rsv	1/32	1/16
8 rsv	1/32	1/16
9 rsv	1/64	1/64
10 rsv	1/32	1/16
11 rsv	1/32	1/16
12 rsv	1/16	1/8
13 rsv	1/16	1/8
14 rsv	1/32	1/16
15 rsv	1/16	1/32

On 15 sera, 13 were concordant according to both methods and 2 were discordant (D)

Evaluation of Parainfluenza Mix

Table 6.

SAMPLE No.	CHORUS	VIRION
1 para mix	1/128	1/256
2 para mix	1/128	1/64
3 para mix	1/64	1/32
4 para mix	1/16	1/16
5 para mix	1/16	1/32
6 para mix	1/64	1/32
7 para mix	1/32	1/16
8 para mix	1/32	1/32
9 para mix	1/32	1/32
10 para mix	1/32	1/64
11 para mix	A/C	neg

10 sera were all concordant according to both methods

The sample 11 para mix showed anticomplementary power (A/C), so it was omitted from the evaluation.

Evaluation of *Mycoplasma pneumoniae*

Table 7.

ID SAMPLE	CHORUS	VIRION	CHORUS IgM	CHORUS IgG
1 myco p.	1/128	1/128	0,2 P	>100 P
2 myco p.	> 1/128	> 1/256	7,8 P	<10 N
3 myco p.	1/64	1/32	0,5 N	>100 P
4 myco p.	>256	>256	8,6 P	58,4 P
5 myco p.	1/16	1/16	0,1 N	>100 P
6 myco p.	NEG	1/16	0,4 N	<10 N D
7 myco p.	1/16	1/16	0,6 N	52,2 P
8 myco p.	1/16	1/16	0,6 N	48,2 P
9 myco p.	1/16	1/32	1,3 P	97 P
10 myco p.	1/128	1/256	Not tested	Not tested

On 10 sera, 9 were concordant according to both methods and 1 was discrepant (D) . 9 samples out of a total of 10 were also characterized by Chorus method ELISA kit for the determination of IgM and IgG antibodies against Mycoplasma Pneumoniae

Evaluation of *Echinococcus*

Table 8.

SAMPLE ID	CHORUS	DIESSE
1 ech	1/64	1/64
2 ech	1/64	1/32
3 ech	1/64	1/64
4 ech	1/128	1/128
5 ech	1/32	1/32
6 ech	1/64	1/128
7 ech	1/128	1/256
8 ech	1/64	1/128
9 ech	1/64	1/64
10 ech	1/64	1/32

The 10 sera tested were all concordant according to both methods

Evaluation of Chorus Method : precision between tests

The control sera of Adenovirus, Influenza A Virus, Influenza B Virus and Syncythial Respiratory Virus were tested in duplicate twice a day during 3 consecutive days The table shows the average and the CV% of the delay time expressed in seconds obtained for each control serum

Table 9..

	Hemolysis time	
Control serum	**Average**	**Cv%**
ADV	344	16,3
RSV	462	15,7

INFA	247	12,3
INFB	525	19

CV% = percent coefficient of variation

DISCUSSION

Manual CFT is still commonly used, but its limitations were overcome by the introduction of automated systems, facilitating its running without losing its peculiar characteristics of sensitivity and specificity. With the Chorus system it has been possible to:

1. reduce the assay time compared to 18 hours of incubation required by the manual method
2. automate testing
3. eliminate subjectivity in the interpretation of results with a standardized and reliable procedure.

The study performed on the evaluation of the results obtained with the Chorus system with those obtained by using the Virion-Serion antigens against a panel of human sera has provided a correlation of 91.6%: 7 sera were discordant out of a total of 83 samples. The two compared methods showed similar results , the ADV discordant results (3/15) may find a possible explanation considering the different composition of the antigens used in both methods. The antigen used on the Chorus contains serotypes 2 and 7, while the serotypes 2, 3, 4 and 7 are used in the Virion-Serion method . . (7). The Chorus CFT method provides good performances in terms of sensitivity, reliability and consistency of results in-run, between-run and among lots. This last figure indicates the high standardization reached in the production and alignment of the Chorus CFT. Its flexibility and cost reduction, since the monotest device contains all reagents needed for one single test, are an added advantage. Precision studies show a good consistency of results; the data are supported by the performance of the control chart registered by monitoring the titre obtained for the influenza A virus control serum during 33 weeks (data not shown). The results obtained fall always within the acceptability range of +/-1 dilution compared to the expected titre.

CONCLUSIONS

The performance of CFT with Chorus automated system has proved good, since it offers reliability and reproducibility of results similar to the manual method.

The performed study has provided results in substantial agreement with each other showing a correlation of 91.6%. This figure appears very comforting considering all advantages arising from the CFT assay with Chorus system. The automated method is easy to use: all information related to sample analysis performance, in addition to reagents, are contained in the device barcode. The test manual handling is minimized: all reagents are, in fact, ready to use since dilution (one of the most common sources of error) is not needed. Moreover, the use of standardized reagents reduces the high variability due to the necessity to titre reagents before use. Even the reporting time is a Chorus's point of force; results, in fact, are provided on the same day when the analysis is run, making this method also suitable for rapid diagnosis, unlike the manual method which gives results in 18/24 hours. 30 different tests based on different methods can be run simultaneously on the Chorus automated system during 2 hours' average length of time. It leads also to a high reduction of the operators' turnaround time. The Chorus system is indeed versatile and suitable for small laboratories, where the daily quantity of performed tests changes numerically in comparison to larger centres. The photometric reading of the haemolytic kinetics and the results processing performed by a dedicated software eliminate subjectivity in the interpretation performed by visual inspection. The management of reagent disposal is made also easier after use.

In conclusion, the automated Chorus system is suitable for performing CFT, either for the reliability of its analytical data and for the level of simplification and standardization of the method, difficult to reach with the manual method.

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