

A comparison study between Enzyme-linked immunosorbant assay and rapid latex agglutination test for the diagnosis of Rotavirus in human

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Received 27 May 2014 ; Accepted 6 July 2014

Abstract

The present study was conducted for the period from 1st/ August/ 2010 to 30th/ November/2011 in Baquba city. The study aimed to explore the diagnostic capability of two laboratory techniques namely; rapid latex agglutination test, enzyme-linked immunosorbant assay for the detection of rotavirus in stool specimens of human. The study included 120 patients with acute diarrhea, 70 were male and 50 were female. The age range was from two months to 5 years. Detection of rotavirus in stool specimens was done by direct agglutination test (DAT) and Enzyme-linked immunosorbant assay (ELISA). The results of present study showed that the overall infection rate by rotavirus among patients with acute diarrhea by DAT and ELISA tests was 70% and 93.33%. The sensitivity and specificity of DAT and ELISA tests in detecting of RV in human stool specimens were 75.56% and 66.67% for the DAT test, whereas, 91.3% and 66.67% for ELISA test. The study concluded that rotavirus was detected in high rates among children less than 5 years old with acute diarrhea in Baquba city, particularly those less than 2 year old. Furthermore, the detection rate by ELISA was higher than by DAT test, suggesting that ELISA is the most appropriate method with excellent

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sensitivity and specificity for detection of RV in stool specimens in a setting like that in Baquba city.

Key wards: Rotavirus, Acute diarrhea, ELISA.

دراسة مقارنة بين تقنية الاليزا و تقنية التلازن المباشر في الكشف عن الفيروس العجلي في الانسان

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الخلاصة

اجريت الدراسة للمدة من 2010/8/1 ولغاية 2011/8/30 في مدينة بعقوبة. وكان الهدف منها التحري عن القدرات التشخيصية لاثنتين من التقنيات المختبرية و هي: تقنية التلازن المباشر و تقنية الاليزا على الكشف عن الفيروس العجلي في نماذج البراز المأخوذة من الإنسان. شملت هذه الدراسة 120 مريضاً ممن يعانون من الاسهال الحاد 70 من الذكور و 50 من الاناث تتراوح اعمارهم بين شهرين الى خمس سنوات. اجري الكشف عن الفيروس العجلي في نماذج البراز بواسطة اختبار التلازن المباشر. أظهرت نتائج الدراسة الحالية ارتفاعاً في نسب الإصابة الكلية بالفيروس العجلي بين المرضى المصابين بالإسهال الحاد و باستخدام اختبار التلازن المباشر و اختبار الاليزا حيث كانت 70% و 93.33% على التوالي. كانت الخصوصية و النوعية لكل من اختبار التلازن المباشر و اختبار الاليزا في الكشف عن الفيروس العجلي في نماذج براز الإنسان (75.56%, 66.67%) بالنسبة لاختبار التلان المباشر, بينما كانت النسب (91.3%, 66.67%) لاختبار الاليزا. تستنتج الدراسة ان نسب الكشف عن الفيروس العجلي كانت عالية بين الاطفال الذين تتراوح اعمارهم دون الخامسة من العمر و يعانون من الاسهال الحاد وخاصة الاطفال الذين هم دون السنتين من العمر في مدينة بعقوبة. علاوة على ذلك ان نسب الكشف بواسطة اختبار الاليزا كانت اعلى من تلك التي كانت بواسطة اختبار التلازن المباشر مما يدل ان طريقة الاليزا الافضل من حيث حساسية و خصوصية للكشف عن الفيروس العجلي في نماذج البراز في مدينة بعقوبة.

الكلمات المفتاحية: الفيروس العجلي, الاسهال الحاد و الاليزا.

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Introduction

Today, Rotaviruses are recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhea in young children in both developed and developing countries worldwide (Bishop, 2009)¹. Although RV can infect older children and adults, diarrheal disease is primarily observed in children under 2 years of age (Bernstein, 2009)². It accounts for 5% of all deaths in children younger than 5 years in developing countries. Mortality rates in developed countries on the other hand are very low and illness is usually self limiting (Black *et al.*, 2010³; Frieden *et al.*, 2011⁴). The RV belongs to the *Reoviridae* family, exhibits icosahedral symmetry, it is not enveloped and was first identified by electron microscopy by Bishop *et al.* (1973)⁵. The viral particle consists of three layers of protein and the viral genome consists of 11 segments of double-stranded RNA (dsRNA), which encode six structural proteins, VP1-4, VP6 and VP7 and six non-structural proteins, NSP1-6 (Pesavento *et al.*, 2006)⁶. RVs are transmitted by fecal-oral spread, and possibly also by the respiratory route (Kapikian *et al.*, 2001)⁷. Initially, diagnosis of rotavirus infection was performed using electron microscopy, by visualization and observation of the rotavirus wheel-like appearance (Arcangeletti *et al.*, 2005)⁸. Nowadays, the laboratory diagnosis of rotavirus infection is usually performed by antigen detection, using latex agglutination techniques, which is affirmed to be a good tool for the simple and rapid detection of RV in stool specimens (Kohno *et al.*, 2000⁹; Pirkooh and Shahrabadi, 2007¹⁰). Enzyme-linked immunosorbant assays which are the most preferably used due to their high sensitivity and specificity for detection of RV in different pathological specimens (Ghazi *et al.*, 2005¹¹; Ferreira *et al.*, 2006¹²; Vainio *et al.*, 2009¹³). RV-induced diarrhea now is considered to be a disease that can be prevented through vaccination (Vesikari, 2009)¹⁴. Two live attenuated oral rotavirus vaccines were licensed in 2006: Rotarix (GlaxoSmithKline), a human rotavirus vaccine with G1P[8] serotype characteristics, and RotaTeq (Merck), a bovine-human reassortant vaccine expressing human G1-4 and P[8] antigens. With the introduction of these two vaccines in many countries, it appears that the total number of

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hospitalizations due to RV infections is being reduced, at least in developed countries that implemented a universal immunization program (Greenberg and Estes, 2009)¹⁵.

Materials and methods

Patients and Methods:

Study groups:

The present study was conducted in Baquba city for the period from 1/August/2010 to 30/August /2011. It included; the group comprises 120 patients with acute diarrhea. The patients were attended Al-Batool Maternity and Children Teaching Hospital and the following Primary Health Care Centers in Baquba (Al-Takea, Al-Sarai and Al-Tahrer). Seventy of patients were males and 50 were females. The age range was 2 months to 5 years.

Collection of stool specimens:

One hundred and twenty stool specimens were collected from patients were diagnosed by the doctor with disposable clean containers with tightly fixed caps. The specimens were transferred to the laboratory of Al-Batool Maternity and Children Teaching Hospital in a cool box as soon as possible. Direct detection of RV in stool specimens using direct agglutination test was performed as soon as possible after collection. The direct agglutination technique was carried out at Al-Batool Maternity and Children Teaching Hospital in Baquba. For ELISA test, about 1 gram of stool specimen was added to 4 milliliters of ELISA diluted washing buffer and the specimens kept frozen at -20⁰ C until used. The ELISA technique was carried out at the Virology Unit/ Public Health Laboratory in Baquba.

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Detection of rotavirus:

Direct agglutination test:

This test was performed using commercially available kit (Diaspot – USA Direct agglutination kit). Positive results were indicated by the appearance of two distinct colored lines. One colored line represents the control red line region and the second colored red line represents the test line region. The Negative results were indicated by the appearance of one distinct colored line in the control region only.

Test procedure:

1. The test was performed within 2 hours after collection following the manufacturer's instructions. The test device, specimens, and buffer were left for 15 minutes in room temperature prior to testing.
2. For solid stool approximately 50 mg were taken randomly by specimen collection applicator, and for liquid stool 2 drops (approximately 50 microliters) were taken. In each case, the specimen was transferred to specimens collection tube containing the dilution buffer and shaken vigorously to homogenize the mixture.
3. Two full drops of the extracted specimen (approximately 80 microliters) were transferred to the specimen well (S) of the test device and then started the timer.
4. The results were read at 10 minutes after dispensing the specimen.

Rotavirus Ag (stool) ELISA test:

This test was performed using commercially available kit (DRG-Germany Rotavirus Ag ELISA stool kit). Reactive results were indicated by the absorbance reading of 0.15 and above, while the non-reactive results were indicated by the absorbance reading less than 0.15.

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Test procedure:

1. One hundred microliters of each of negative control and positive control were added to assigned wells (used both as undiluted).
2. One hundred microliters of the stool supernatant was added to the appropriated test well (patient wells).
3. The plate was incubated at room temperature for 30 minutes, and then washed for 3 times with diluted washing buffer.
4. Two drops of anti-rotavirus antibodies were added to each well.
5. The plate was incubated at room temperature for 5 minutes, and then washed for 3 times with diluted washing buffer.
6. Two drops of anti-mouse antibodies conjugated to horseradish peroxidase were added to each well.
7. The plate was incubated at room temperature for 5 minutes, and then washed for 3 times with diluted washing buffer.
8. Two drops of Chromogen was added to each well.
9. The plate was incubated at room temperature for 5 minutes.
10. Two drops of Stop solution were added to each well. Mixed wells by tapping strip holder.
11. The optical density (OD) of wells was read using ELISA spectrophotometer reader at 450 nanometers wave length.

Statistical analysis:

Data analyses was computer aided. Statistical analysis were done using SPSS (Statistical Package of Social Science) version 18 computer software. Frequency distribution and

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percentage for selected variables were done first. The validity of screening tests was examined by evaluate the sensitivity and specificity of tests (Niazi, 2004)¹⁶. Whenever we examined the validity of a screening tests, we must compare the results with a confirmatory or referral test.

Results and Discussion

Results presented below are based on statistical analyses of the data accumulated throughout the study period.

Detection of rotavirus in human

Direct agglutination test

Results in table (1) revealed that RV was detected in 84 (70%) of patient stool specimens, while 36(30%) of the specimens were negative.

Table (1): Results of DAT test for the detection of rotavirus in human stool specimens

Result		Frequency	%
Human n=120	Positive	84	70.0
	Negative	36	30.0

The present results were inconsistent with other Iraqi researchers (Younis, 1989¹⁷; Al-Falahi, 2002¹⁸; Hasan *et al.*, 2011b¹⁹) who reported an infection rate of 26.3%, 24.4% and 20.3%, respectively among children less than 5 years using DAT technique. Nevertheless, worldwide studies have yielded variable RV infection rates among children less than 5 years old; for instance, in Korea (Koh *et al.*, 2008)²⁰ reported an infection rate of 41.3%, while in Saudi Arabia, the infection rate was 30% (Kheyami *et al.*, 2006)²¹. These controversies in the

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results of the different studies maybe attributed to many reasons, the difference in geographical area, seasonal variation, sample size of the study, and the concentration of RV antigen in the collected specimens. However, the present study suggest that the latex agglutination test for detection of RV antigen in stool specimens is a good tool for the simple and rapid screening test in a limited resources settings.

Enzyme-linked immunosorbant assay

Using the ELISA technique for detection of RV in stool specimens, the results showed that 112(93.33%) of the specimens were positive, and only 8 (6.67%) were negative, table (2).

Table (2): Results of ELISA technique for the detection of rotavirus in human stool specimens

Results		Frequency	%
Human n=120	Positive	112	93.33
	Negative	8	6.67

In the present study, RV was detected in 93.33% of stool specimens by ELISA technique. Since, worldwide studies had reported variable results, so the present results were in agreement with some of these studies, like that conducted in Mecca-Saudi Arabia which reported an infection rate 95.8% among infants and young children with acute diarrhea (Chazi *et al.*, 2005)¹¹. However, it was higher than that reported by other studies; for instance, in Al-Karak Jordan, RV was detected in 39.9% of stool specimens of children below 5 years of age (Nafi, 2010)²². In European children younger than 5 years of age, the infection rate was 43.3 % (Forster *et al.*, 2009)²³. The disparity in the results of the different studies maybe attributed

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to the viral stool load, the sensitivity and specificity of ELISA technique employed, and the technical conditions.

Sensitivity and specificity

The sensitivity and specificity of DAT and ELISA in detecting RV in human stool specimens were shown in table (3). The sensitivity, specificity, positive predictive and negative predictive values of DAT was 75.56%, 66.67%, 97.22% and 15.39% respectively. Whereas, the sensitivity, specificity, positive predictive and negative predictive values of ELISA was 91.3%, 66.67%, 97.67% and 33.33% respectively.

Table (3) : Sensitivity and specificity of DAT test and ELISA test in detection rotavirus in human patients

Detection Methods	Sensitivity	Specificity	+ve predictability	-ve predictability
DAT	75.56%	66.67%	97.22%	15.39%
ELISA	91.3%	66.67%	97.67%	33.33%

The present study revealed that the sensitivity and specificity of DAT test 75.56%, 66.67% respectively. These results were consistent with other researcher reported sensitivity and specificity for latex agglutination test 82.6%, 81.6% respectively (Pereira *et al.*, 2011)²⁴. However, other worker reported higher result, the sensitivity and specificity were 98.2%, 94% respectively (Kohno *et al.*, 2000)⁹. The present results were agreement with results of other worker that the DAT technique is a good tool for the simple and rapid detection of RV in stool specimens (Kohno *et al.*, 2000⁹; Al-Yousif *et al.*, 2001²⁵; Pirkooh and Shahrabadi, 2007¹⁰). Other importance result was reported in this study, the sensitivity and specificity of ELISA test 91.3%, 66.67% respectively. The present results were in agreement with results of other worker that the ELISA technique a highly beneficial sensitive and accurate for detection

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of RV in stool as well as other pathological specimens (Fischer and Gentsch, 2004²⁶; Chazi *et al.*, 2005¹¹; Vaino *et al.*, 2009¹³).

Detection rate by different techniques

Table (4) showed a comparison in the detection rate of the 49 stool specimens tested by the three different techniques. The positivity rate of DAT, ELISA, and RT-PCR were 73.5%, 87.8%, and 93.9% respectively. The detection rate of RT-PCR was found to be significantly higher ($p=0.01$) as compared to other two techniques.

Table (4): Rotavirus detection rate by the three techniques

Tests	No. positive (%)	No. negative (%)	P value
DAT	36(73.5)	13(26.5)	0.015
ELISA	43(87.8)	6 (12.2)	

Detection rate by different techniques

The present study revealed that the DAT and ELISA tests were highly significant difference when they compared to RT-PCR test in detection of RV in stool specimens. Other researcher also reported differences among diagnostic techniques for detection of RV in stool specimens, (Vainio *et al.*, 2009)¹³ used ELISA, DAT, and RT-PCR techniques for detection of RV in stool specimens, the detection rate was 63%, 58%, and 72% respectively. Additionally, (Wible *et al.*, 1991)²⁷ reported an infection rate by RV of 96 stool specimens for children < 5 years of age with acute diarrhea 36% by ELISA test, while 58% by RT-PCR test. The disparity among results of three tests (DAT, ELISA, and conventional RT-PCR) may be due to many reasons, imported, Volume of specimen collected that the lower virus concentration may be leading to classification of such as cases as negative. Additionally,

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the sensitivity and specificity for three techniques, and technical conditions. The present results indicate the importance of using ELISA and RT-PCR technique for detection of RV in stool specimens, and this agreement with other studies (Buesa *et al.*, 1996²⁸; Husain *et al.*, 2010²⁹) because of the RT-PCR is generally considered the gold standard tool in detection of RV and ELISA was found to be as effective as RT-PCR, additionally, it was a highly sensitive and accurate for detection of RV in stool specimens (Jarasvech *et al.*, 1993³⁰; Xiaoli *et al.*, 2004³¹; Logan *et al.*, 2006³²).

Conclusions

1. RV is found to be responsible for high proportion of acute diarrhea in Baquba city among children below 5 years, particularly those less than 2 year of age.
2. Both ELISA and DAT tests have shown good level of sensitivity and specificity in detection of RV in stool specimens.

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