Thesis on clinical trials of Refluvac influenza inactivated whole-virion aluminum vaccine (A/H1N1v type)

**Trial name:** Randomized blind placebo-controlled Phase II on single application of Refluvac (influenza inactivated whole-virion aluminium vaccine on volunteers of aged 18-60 years.

**Clinical trial of registration site, if registered** (e.g. ClinicalTrials.gov): not registered.

**Authors/sponsors:** Republican governmental enterprise on the basis of economic control rights of “Research Institute for Biological Safety Problems” of the Science Committee of Ministry of Education and Science of the Republic of Kazakhstan. Research Plan (including of phase of the clinical trials):

Randomized blind placebo-controlled Phase II on single application of Refluvac (influenza inactivated whole-virion aluminium vaccine) with dose increase on volunteers of aged 18-60 years was carried out. Volunteers have antibodies titer ≤1:10 to subtype of influenza A/H1N1v virus detected by HAIT and seronegative to HIV, C and B types of hepatitis by ELISA data were included in this research. 80 of healthy volunteers are included in research. Volunteer’s vaccination by Refluvac preparation was carried out in the regime of two levels of doses (in doses 3.75 µg or 7.5 µg of hemagglutinin (HA) per person 0.5 ml). One volunteer in the group vaccinated by 7.5 µg of HA (clinical base of Research Institute of Influenza) was excepted because he has strong undesirable phenomena non-connected with vaccination. So, primary and the second tasks of researches were included data the obtained by 79 vaccinated volunteers (40 of volunteers were vaccinated in the dose of 3.75 µg of HA and 39 - 7.5 µg of HA). Research regulation is presented in the table 1.

Table 1. Regulation of the clinical study

<table>
<thead>
<tr>
<th>Procedures/Observation days</th>
<th>Screening</th>
<th>1 (basic rates)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>21 day ± 2 day</th>
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<tbody>
<tr>
<td>Receiving informed consent</td>
<td>X</td>
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<tr>
<td>Control of vital symptoms rates</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Physical examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
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<td>X</td>
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<td>Neurological examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>X</td>
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<tr>
<td>Laboratory study:</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
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<td>Clinical and biochemical blood analysis</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Tests for HIV, B and C types of hepatitis</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Urine analysis</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>X</td>
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<td>Urine test for pregnancy (women of reproductive age)</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Electrocardiogram</td>
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<td>Introduction of the studied preparation</td>
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<td>Sampling for the assessment of immune response</td>
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<tr>
<td>Identification of antibody titers in blood serum by the method of HAIT</td>
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<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Identification of antibody titers in blood serum by the method of micro neutralization reaction</td>
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<td>Adverse reactions and concomitant treatment</td>
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<td>X</td>
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</table>

<sup>a</sup> Before vaccination. <sup>b</sup> After inoculation of tested medical preparation: in 20 minutes and in 2 hours after vaccination.

The vaccine was inoculated in the first day of observation. Active observation of volunteers was conducted within 7 days after vaccination (in vaccination day and in the following 6 days). In the
further, beginning from 7 through 21 days of observation volunteers were kept a self-observation diaries, where they registered appearance of any symptoms and all taken medical preparations. Study was carried out in the clinic of Research Institute of Influenza of Ministry of Health and Social Development of Russia and (outpatients’) clinic of the Mechnikov Research institute of Vaccines and Sera, and was two-centre study. Observation report WRC-II-00-03/2010 version 01 was dated by October 30, 2010. Insignificant amendments were inserted into report, which had been endorsed by the Sponsor and registered in writing.

All duration of participation of every volunteer in researches are no more 21±2 days. Studies were included screening, visit of initial level, 2-7 visits and the final eighth visit on 21 day of study. Interval between screening visit and the initial level of visit was no more 14 days and between the initial and the final visits were 21±2 days.

**Vaccine subtype:** pandemic influenza A/H1N1v  
**Virus:** NIBRG-121xp (NIBSC, Great Britain). Vaccine strain was obtained by reverse genetic method and contains HA and NA genes of influenza A/California/7/2009 (H1N1)v virus and PA, PB1,PB2, NP, M and NS genes from high-yield A/PR/8/34 strain.  
**Producer:** Republican governmental enterprise on the basis of economic control rights of “Research Institute for Biological Safety Problems” of the Science Committee of Ministry of Education and Science of the Republic of Kazakhstan.

**Type (whole virus/subvirion/subunit/live/recombinant/DNA/vector):** whole virion inactivated  
**Adjuvant:** an aluminium hydroxide  
**System/place of inoculation:** intramuscularly  
**Doses (antigen and adjuvant, amount of doses, interval between inoculations):** Refluvac vaccine was inoculated single intramuscularly (in deltoid muscle; 0,5 ml) in doses 3.75 µg HA (Al⁺³ 0,125 mg) or 7.5 µg HA (Al⁺³ 0,25 mg) per person.

**Study on population**  
**Subject’s number:** 80 volunteers  
**Age range:** 18-60 years  
**Health status:** healthy volunteers  
**Special access criteria/dropout**  
**Inclusion criteria:**  
- healthy volunteers, male and female, aged 18-60 years;  
- volunteers who are seronegative to A/H1N1 influenza virus (with antibody titers 1:10 according to HAIT);  
- written acceptance from volunteers to participate in this study.

**Exclusion criteria:**  
- Allergic reaction to chicken protein or any of the preceding vaccination.  
- Acute illness with fever (>37,0°C).  
- History of chronic abuse of alcohol and/or narcotic;  
- Severe form of atopy in history.  
- Vaccination against influenza A/H1N1(participants of the clinical studies of H1N1 vaccine in 2006-2007).  
- Volunteers with clinically significant abnormal laboratory signs.  
- Women with affirmative result of pregnancy.  
- Simultaneous taking of immunosuppressive drugs, including corticosteroids, (≥2 weeks) in the period of 4 weeks before the introduction of the studied preparation.
The presence of clinically important lesions of the kidneys, liver, gastrointestinal tract, cardiovascular system, blood system, skin, endocrine, neurological or immunological diseases.

- The presence of leukemia or malignant tumors.
- Persons who are seropositive to HIV or B or C types of hepatitis.
- Volunteers, who took antiviral drugs, immunoglobulins or blood transfusion or any other drug in the period of 4 weeks before the introduction of the investigational preparation.
- Volunteers, who received anti-inflammatory drugs for 2 days before the introduction of the investigational preparation.
- Participation in any other clinical studies during the last 3 months.
- Volunteers, who may not comply with the requirements of study, or persons with severe physical or mental disabilities that may have an impact on the completion of the study.

Available clinical final item

Reactogenicity and safety assessment: Unwanted cases (local and systemic reactions), data of physical examinations, vital symptoms, the results of laboratory studies (general and biochemical blood tests, IgE, common urine analysis), electrocardiogram.

Evaluation immunogenicity/effectiveness: Humoral immune response (levels of seroconversion and seroprotection, GMT, factor of serocversion) - with chicken erythrocytes by HAIT. Cellular immune response – by stimulation index and cytokines production in supernatant antigen stimulated cells.

Results

Reactogenicity and safety:

Serious objectionable phenomena and objectionable phenomena of strong level connected with vaccination were not observed. Volunteers inoculated by Refluvac vaccine in dose 3.75 µg HA systemic reaction connected with vaccination was not observed. 10 from 40 inoculated volunteers (25%) had local reaction in weak level as pains and “discomfort” and was not attended by the development of hyperemia or infiltrates, had transient character (no more than 2 days) and disappeared without use of drugs. Among volunteers inoculated by Refluvac vaccine in dose 7.5 µg HA was noted one systemic reaction of average level (rise temperature up to 37.8 °C) on the first and the second days of vaccination. 6 from 39 inoculated volunteers (15,4%) had local reaction in weak level as pains and “discomfort” and was not attended by the development of hyperemia or infiltrates, had transient character (no more than 2 days) and disappeared without use of drugs. Result changes of clinical laboratory observations of volunteers on 7 days and 21 days after vaccination from the primary rates including general level IgE occurred within normal physiological significances regardless of vaccine dose. Negative vaccination influence on the electrocardiogram data was not detected, after vaccination the electrocardiogram data was stayed on the level of the primary values.

Immunogenicity:

Evaluation of immunogenic activity of vaccine for single inoculation (with chicken erythrocyte and RDE) by HAIT data within 21 days after vaccination was showed that in the group of inoculated by Refluvac vaccine in dose 3.75 µg HA with 4-multiple seroconversion was 85,0%; the level of seroprotection was 85%; increase multiple antibodies titres was 14,4; the geometric mean titres (GMTs) of antibody to influenza A/H1N1v virus was 100,5. In the group of inoculated by Refluvac vaccine in dose 7.5 µg HA was noted increasing of all rates of immunogenic activity of vaccines: 4-multiple seroconversion was 92,3%; GMT and increase multiple antibodies titres were 175,5 and 27, respectively; the level of seroprotection was 92,3%. Study of immunogenic activity of Refluvac preparations in doses 3.75 µg and 7.5 µg HA were that vaccine after single inoculation
has immunogenic activity and meet of all European requirements CPMP EMEA and criteria of Federal Service of supervision in sphere of protection of the rights of consumers and well-being of the person MU 3.33.1758-03 for seasonal inactivated influenza vaccines. Vaccination of Refluvac preparation produced to formation of cellular immune response with polarization of section Tx-1.

**Conclusion:** Study of immunogenic activity, reactogenecity and safety of Refluvac vaccine in doses 3.75 µg and 7.5 µg HA of single intramuscular immunization of volunteers aged 18-60 years were shown that vaccine has immunogenic activity and meet of all European requirements CPMP EMEA and criteria of Federal Service of supervision in sphere of protection of the rights of consumers and well-being of the person MU 3.33.1758-03 for seasonal inactivated influenza vaccines, low reactogenecity and vaccine safety. Vaccination of Refluvac preparation produced to formation of cellular immune response with polarization of section Tx-1. The obtained results allow recommending Refluvac vaccine in dose 3.75 µg HA for the state registration as the prophylactic means for protection of the population at the age of 18 - 60 years from A subtype of the H1N1 influenza.

**State of the clinical trial on this moment (completed, carrying out, in the phase of preparation):** Evaluation of safety tolerance of Refluvac vaccine was completed I and II Phases

**Starting time of the next phase of the development by schedule:** Next phase of development is the state registration Refluvac vaccine to the Committee of Pharmacy of the Ministry of Health of the Republic of Kazakhstan.