# IRN AR23489672 “A Novel Phage-Based Biological Product for Combating Treponema pallidum”

*Project Leader: Syrym N.S..*

## Annotation

The project is aimed at developing a bacteriophage-based therapeutic agent against Treponema pallidum, the causative agent of syphilis. Phage therapy is proposed as a safe and effective treatment method with minimal side effects compared to antibiotics. The project outcomes are expected to improve syphilis treatment in Kazakhstan and facilitate the implementation of innovative therapeutic approaches.

## Purpose and objectives

- ✓ The research involves the isolation of Treponema pallidum spirochetes from syphilis-infected patients and their molecular-genetic typing.
- ✓ Biological and molecular-genetic characterization of the isolated bacteriophages targeting Treponema pallidum will be performed.
- ✓ A phage preparation against T. pallidum will be developed, followed by assessment of its therapeutic efficacy.

## Methods and materials

- ❖ **Cultivation** — Treponema pallidum propagation in male rabbits (in vivo) and Sf1Ep cell lines (in vitro).
- ❖ **PCR and sequencing** — Molecular-genetic typing of Treponema pallidum based on the tpp47 gene.
- ❖ **IFA**— Detection of antibodies against Treponema pallidum.
- ❖ **Phage isolation** — Enrichment culture method using clinical material from patients.
- ❖ **Phage evaluation** — Assessment of lytic activity (Appelmans and Gratia methods), specificity (Otto method), thermal stability, chemical resistance (chloroform), as well as PCR and sequencing for molecular characterization.

## Results and discussion

- ✓ Clinical samples were collected with prior informed consent and approved by the relevant Human Research Ethics Committee.Detection of specific antibodies against Treponema pallidum was performed using enzyme-linked immunosorbent assay (ELISA) and the Wassermann reaction.
- ✓ Presumptive Treponema isolates were propagated in adult healthy male rabbits with well-developed, firm, and elastic testes. These animals had not received any antibiotic treatments (either therapeutic or dietary) before or after infection. The rabbits, aged approximately 3 months and weighing between 2.5 and 3.0 kg, tested seronegative in VDRL/RPR and RW (Wassermann) assays in accordance with the manufacturer's instructions.
- ✓ For infection, rabbits were restrained using soft leather straps in a specialized frame. A volume of 1.5 mL of the clinical sample was injected into each testis. In total, 17 rabbits were infected. Clinical monitoring, including thermometry and testicular palpation, was carried out three times per week.
- ✓ After 45 days, signs of orchitis were observed in the testes of infected rabbits. Seropositivity was confirmed via screening using VDRL/RPR and RW tests, which yielded positive results.
- ✓ The obtained biological material was transferred onto a microscope slide, covered with a cover slip (crush preparation), and examined using dark-field microscopy.

1

2

**Fig. 1,2- Infection of rabbits with Treponema pallidum was carried out by introducing 1.5 ml of the sample into the testicle of each of 17 animals. Observation included thermometry and palpation of the testicles three times a week..**

Contacts: 8(72636)7-22-28