

Evaluating Seroconversion Accuracy Compared to HSV Infection Markers



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INTRODUCTION

- Herpes Simplex Virus 2 (HSV-2) is a lifelong infection characterized by symptomatic disease with acute and recurrent genital lesions or asymptomatic infection with no genital lesions
- A HSV-2 vaccine to prevent disease and transmission to partners and newborns is under development
- Serologic tests (antibody assays to measure infection) are one way to evaluate if humans are infected with HSV-2
 - The accuracy of serology tests has been validated in HSV-2 naïve (unvaccinated) individuals but not well-studied in vaccinated individuals
- Currently, the best criteria to measure HSV vaccine performance in humans is to look for lesions and seroconversion

HYPOTHESIS AND QUESTIONS

Hypothesis: Serologic changes are not accurate in those who are vaccinated (many false + and false - results)

- Small amount of virus can stimulate immune response (seroconvert), but not enough to get infected (measured by genital lesions or viral shedding of HSV DNA)
- **What is the most accurate way to determine if someone is infected with HSV in humans?**
 - **Is a change in serology an accurate indicator of infection in those who are vaccinated?**

STUDY DESIGN

- Guinea pigs are a useful model to evaluate genital infection
- 3 groups of guinea pigs used: **1) Unvaccinated (naïve) + uninfected 2) Unvaccinated + infected 3) Vaccinated then infected**
- Markers of infection measured in guinea pigs: **1) serology 2) genital lesions 3) viral shedding of HSV DNA (as indicator of recurrent infection) 4) day 2 & 4 vaginal viral titers post infection 5) HSV DNA in dorsal root ganglia (DRG) - site of HSV latency**
- Evaluate combinations of infection markers measured in guinea pigs vs. best possible criteria (lesions, viral shedding, day 2 & 4 viral titers, HSV DNA in DRG) to evaluate infection
 - **Can determine if including serology will improve or worsen false + and - rates compared to best possible criteria ("gold standard")**

SEROLOGIC METHODS

- **Evaluate serostatus of naïve uninfected (n=12), unvaccinated and infected (n=31) and vaccinated (n=97) guinea pigs challenged with HSV by gG2 ELISA**
 - Use paired sera → serum after immunization + before infection (pre) and terminal bleed serum (post infection)
- **Criteria for positive + negative serology results**
 - ≥ 4 fold titer rise between pre and post sera
 - Confirm any seronegative or indeterminate results (<4 fold titer rise between pre and terminal sera) with western blot
 - BOTH assays must be negative for animal to be considered seronegative

OTHER INFECTION CRITERIA EVALUATED METHODS

- Acute & recurrent infection cumulative lesion days
 - Measured for ≥ 48 days
- HSV DNA viral shedding in vaginal secretions by qPCR
 - Measured for ≥ 20 days
- HSV vaginal viral titers at day 2 and day 4 post-challenge
- Lumbosacral DRG measured by qPCR on virus tissue at end of experiment
 - To detect latent infection
- **If any one is positive, considered infected**

RESULTS

Table 1: Summary of infection criteria in unvaccinated (naïve) + uninfected control animals

	Positive	Negative
Lesions	0	12
Shedding	0	12
Day 2 or 4 titers	0	12
DRG	0	12
Serology	0	12

Conclusion: Naïve uninfected animals are negative for all markers of infection. No false + or false -

****For table 2 and 3, looking at markers of infection in guinea pigs that can be measured in humans and comparing them to the "gold standard" in guinea pigs, which gives most accurate assessment of infection. ("gold standard" criteria noted in Study Design)**

Table 2: Comparing various markers of infection against "gold standard" for unvaccinated + infected guinea pigs

	False Positive Rate	False Negative Rate
Lesions + Serology	0	14.3
Shedding + Lesions	0	10.7
Shedding + Lesions + Serology	0	10.7
Serology	0	21.4

Conclusion: Serology performed well for unvaccinated + infected animals. No false + and only a few false - (~10.7-21.4%)

Table 3: Comparing various markers of infection against "gold standard" for vaccinated + infected guinea pigs

	False Positive Rate	False Negative Rate
Lesions + Serology	14.8	34.3
Shedding + Lesions	0	28.6
Shedding + Lesions + Serology	14.8	34.2
Serology	14.8	45.7

Conclusion: Serology did not perform well for vaccinated + infected animals. Many false + and -

OVERALL CONCLUSIONS

- **Serology tests are more accurate in unvaccinated + infected animals than in vaccinated + infected animals**
 - These inaccuracies should be considered if used in vaccine trials
- **Evaluating lesions + HSV DNA viral shedding = best indicator of infection**
 - However, it is difficult to perform viral shedding experiments in human vaccine trials