

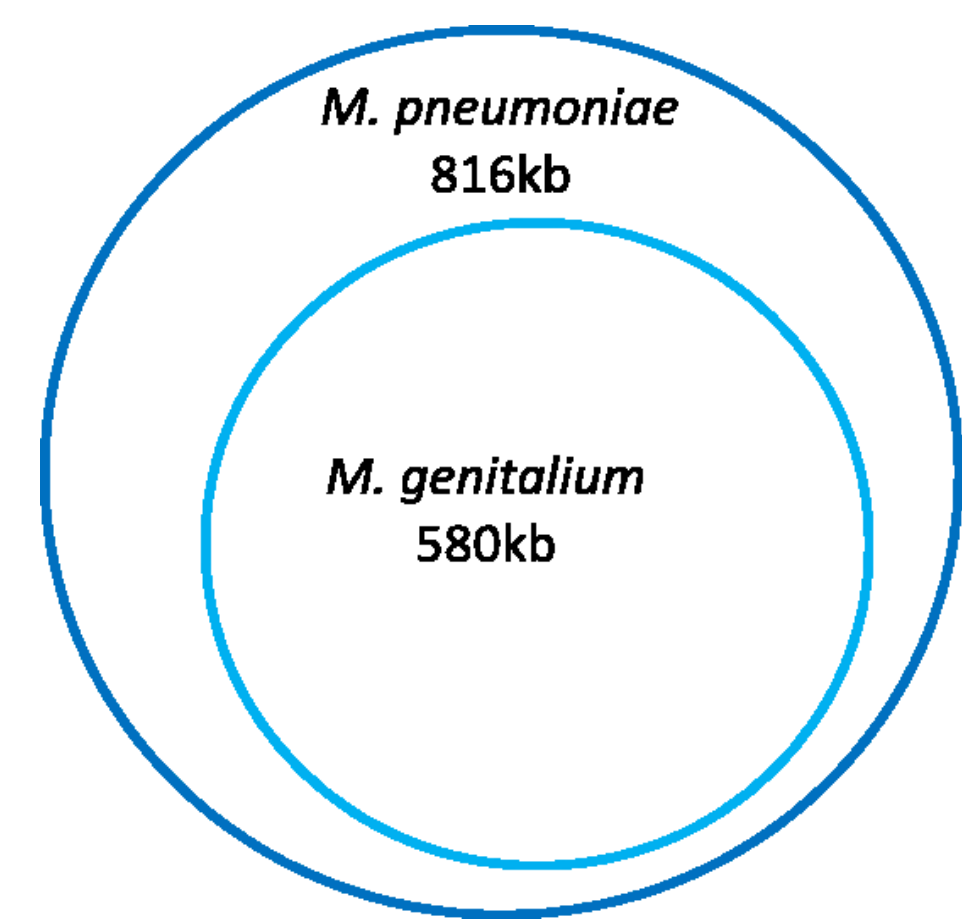
Development of a serological assay for differentiation of *Mycoplasma genitalium* and *M. pneumoniae* infections

Anna Overgaard Kildemoes, Olivia Sureya Seierø Rai, Elisabeth Probst Lyng, Henrik Bekkevold Johansen, and Jørgen Skov Jensen
Reproductive Microbiology, Department of Bacteria, Parasites & Fungi, Statens Serum Institute, Denmark

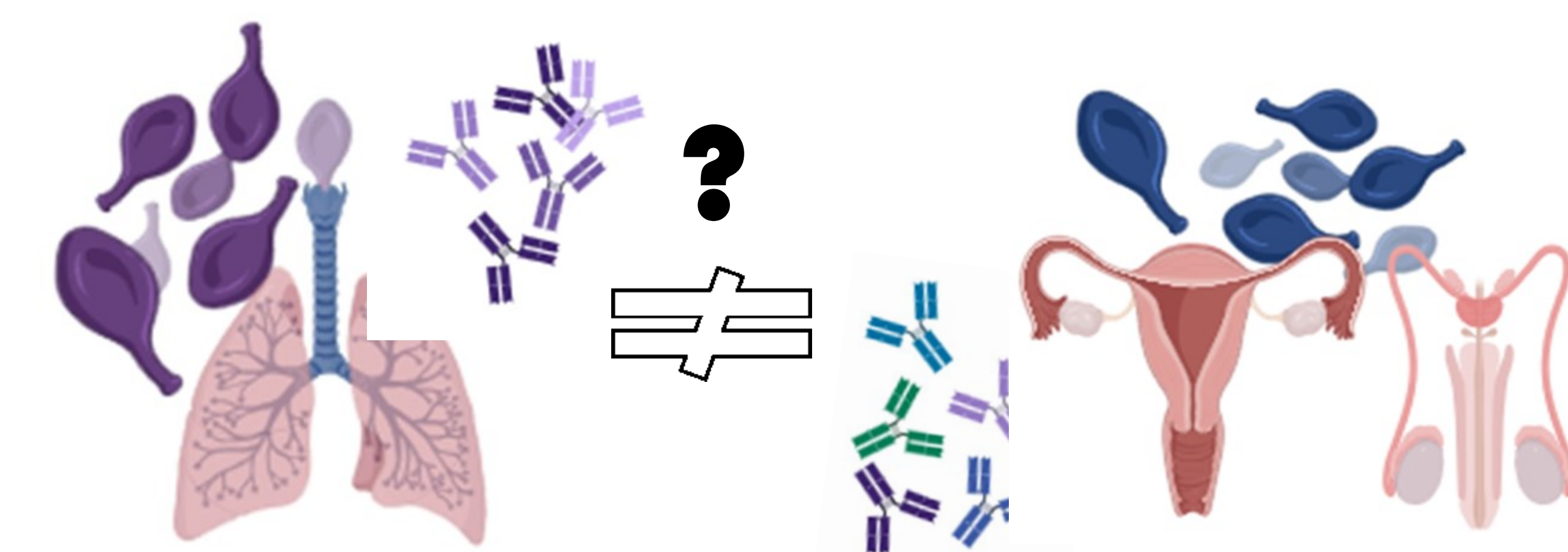
WHY

do we need an assay detecting antibodies specific for *M. genitalium* infection?

M. genitalium (Mg) is a sexually transmitted pathogen, which can cause a range of mild to severe reproductive tract disease and morbidities in both men and women. Single and/or multiple exposures to sexually transmitted infections can lead to complex and persistent local inflammatory responses with **long-term reproductive consequences such as infertility and ectopic pregnancies**. For *Mycoplasma* species excellent molecular methods for detection of current infections and evaluation of treatment efficacy exist. However, **highly specific** serological assays are needed for epidemiological studies at the population level to understand risks and consequences of previous exposures.



All predicted open reading frames in the *Mg* genome have homologs contained within the slightly larger genome of the very common and closely related respiratory pathogen *M. pneumoniae*.
Major challenge = identify highly Mg SPECIFIC polypeptides or protein fragments that generate IgG responses in infected people

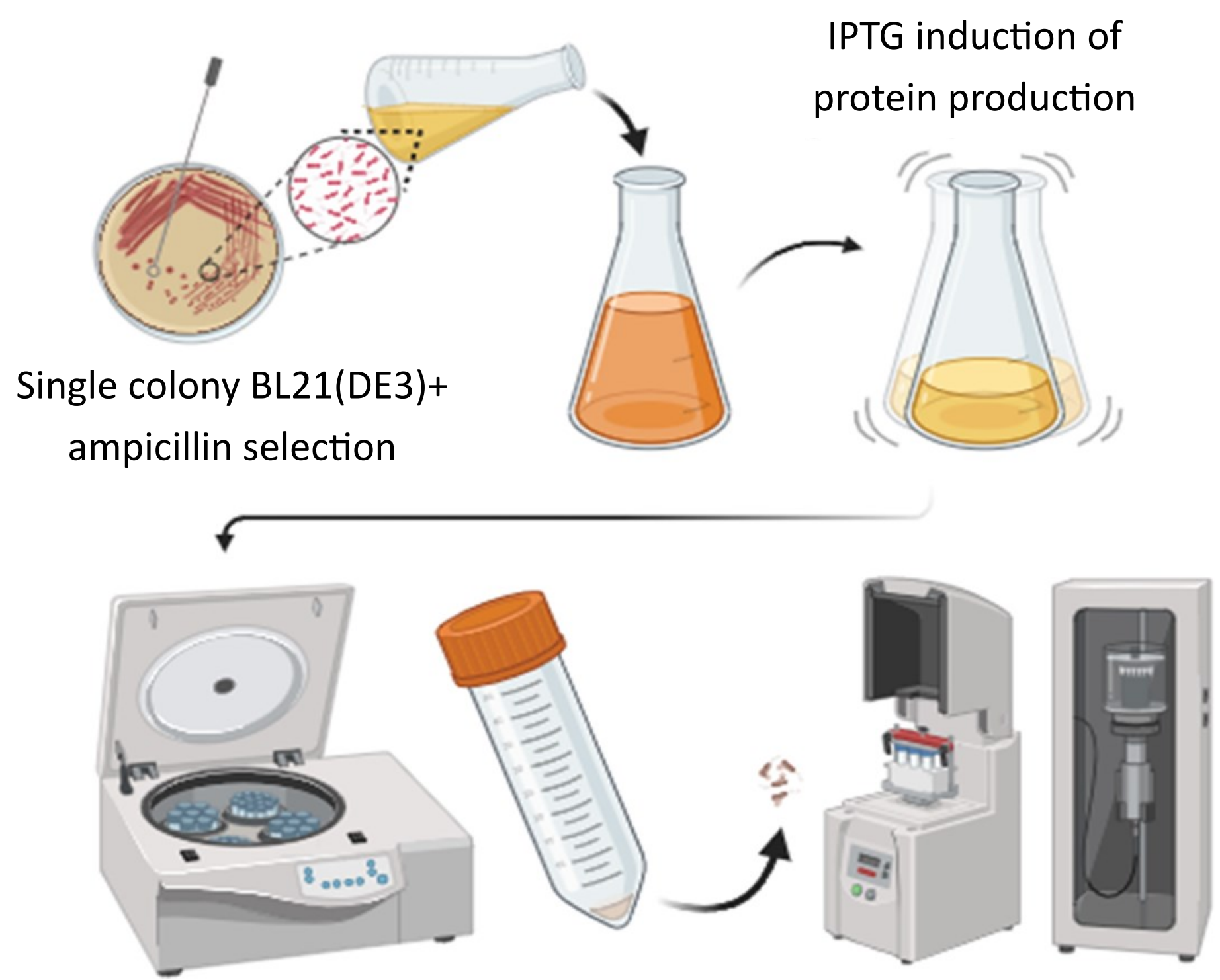


Development of a serological assay, that is capable of distinguishing *Mg* and *Mp* with high accuracy, will enable better understanding of associations between *Mg* and later adverse reproductive events. Such knowledge can be used to inform treatment and prevention strategies in distinct risk populations.

METHODOLOGY

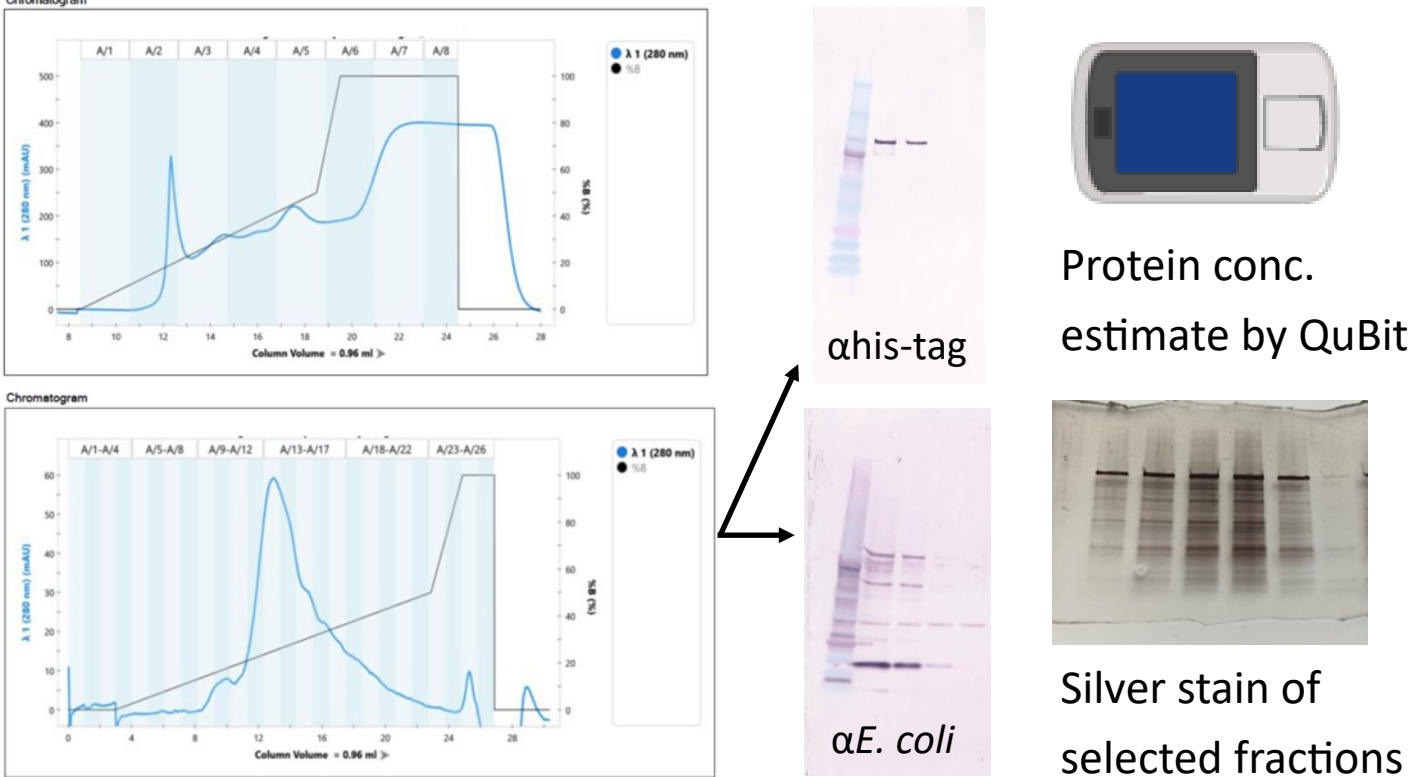
- * Sequences for *Mycoplasma genitalium* protein fragments of interest were cloned into IPTG inducible pET102D/TOPO vectors with ampicillin resistance and His-tag (Invitrogen).
- * Expression was done in standard *Escherichia coli* BL21(DE3) cultures in LB with ampicillin.
- * Purification was done by fractionation on medium-pressure liquid chromatography NGC system first on His-Trap column followed by anion-exchange on a HiTrap column (BioRad).
- * Presence/absence of the protein(s) of interest in fractions was determined by western blotting using anti-his-tag antibodies.
- * *E. coli* derived proteins in fractions were detected with anti-*E. coli* antibodies on western blots (and silver staining of gels).
- * Specificity and sensitivity of protein-fragment candidates were assessed on Western line-blots using known *Mg* and *Mp* positive and negative samples

Protein production:



Pellet harvested at 2500g and cold lysis done by mechanical bead-beating followed by sonification. Respectively, supernatant for soluble products or pellet for inclusion body protein fragments were harvested after centrifugation at 22000g.

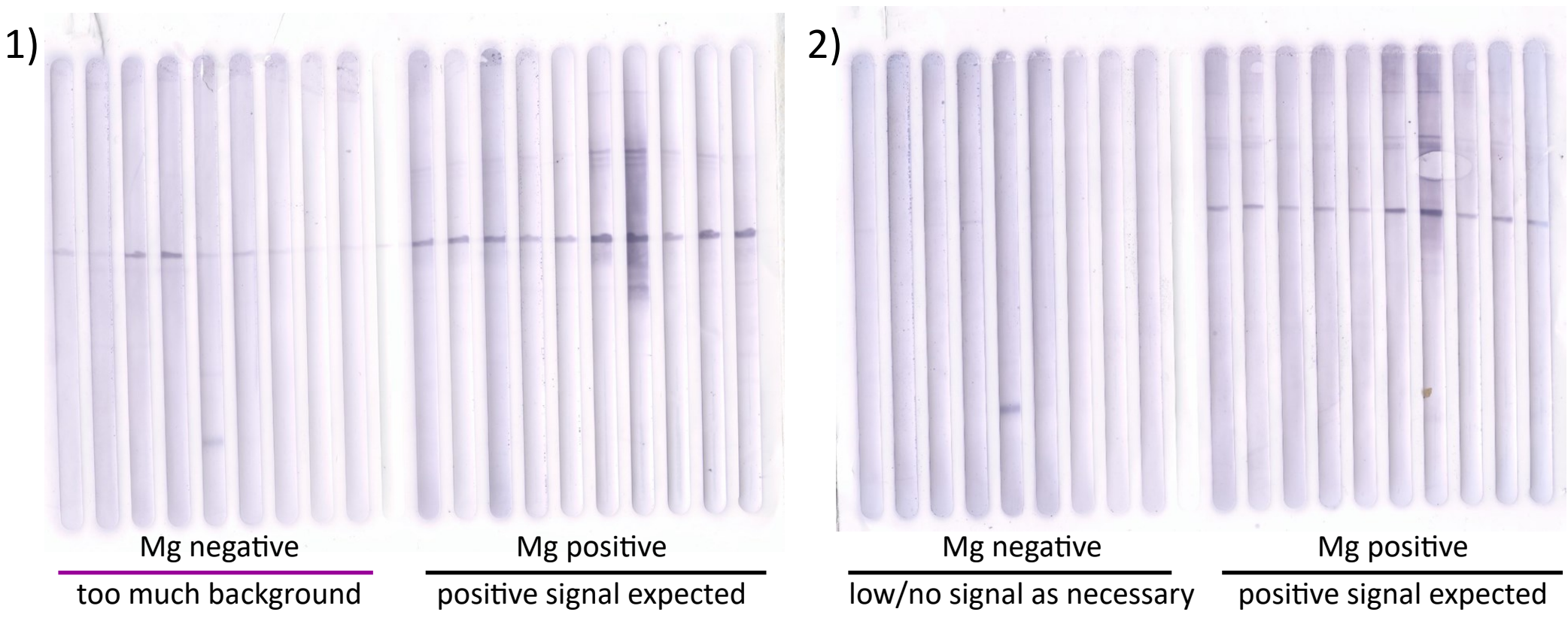
Protein purification:



WORK IN PROGRESS

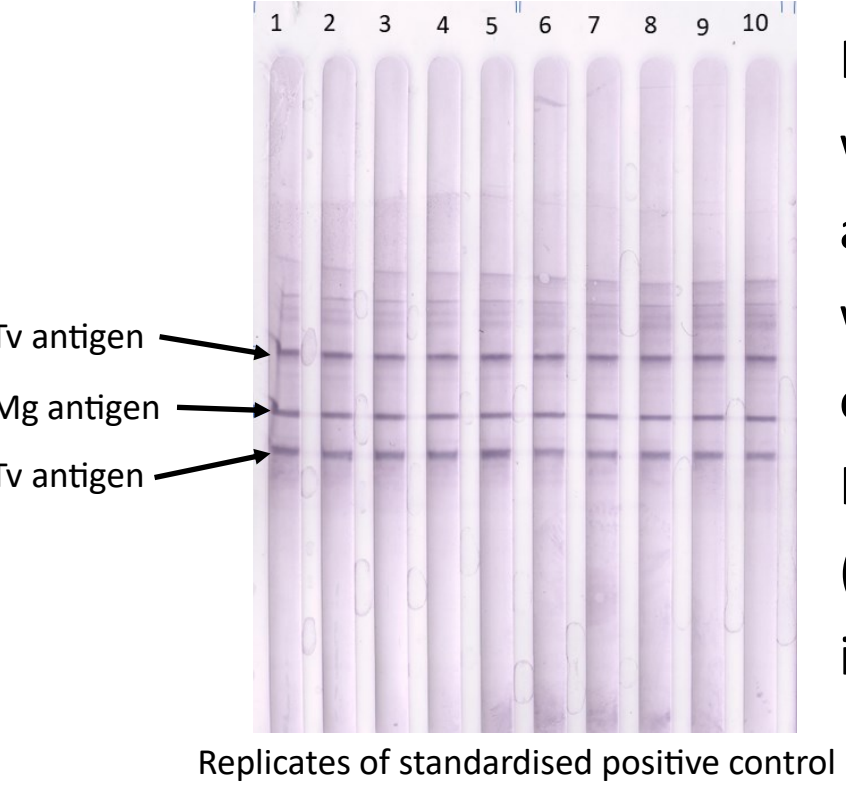
Titration criteria of target antigen to optimal level based on well-characterised samples also used for evaluation of target positive/negative cut-off:

- * *Mg* PCR and/or serology positive samples -> good signal
- * *Mg* assumed negative samples from children ≤15 years -> very low to no signal (highly unlikely to have had *Mg* infection)
- * *Mp* serology positive samples from children ≤15 years -> low to no signal
- * *Mp* serology negative samples -> very low to no signal



Representative lineblots with different antigen concentration but identical samples. Antigen concentration on blot 2 chosen. Evaluated on n > 70.

Standardisation of positive control made from pool of known positive samples here multiplexed with *Trichomonas vaginalis* (Tv) antigen to demonstrate multiplex capacity:



In preliminary experiments, subjective band intensity was determined by direct observation. Image analyses and within blot normalisation to known positive controls will be applied going forward. White light images are obtained on GBOX and relative quantification of specific IgG binding is determined with GeneTools software (Syngene). Reproducibility testing is performed in both intra- and interassay format.

Preliminary specificity and sensitivity in different sample sets: subjective scoring

- SENSITIVITY:** Adults known *Mg* PCR positive (n=66) -> 97% (clinic recruitment)
Adults known *Mg* PCR positive (n=20) -> 85% (field recruitment)
- SPECIFICITY:** Children <15 years old *Mp* serology negative (n=90) -> 90%
Children <15 years old *Mp* serology positive (n=30) -> 73%

Image analyses will be used to standardise cut-off and provide objective measures. Cut-off will be optimised to prioritise specificity and sample sizes increased.

PRELIMINARY CONCLUSIONS

★ A single protein fragment from *M. genitalium* performs with promising specificity for *M. genitalium* compared to known *M. pneumoniae* positive and negative samples. The goal is an overall assay performance of >90% sensitivity and specificity.

ENVISIONED USE CASE of IMMUNOBLOT ASSAY:

Population level epidemiological studies to disentangle consequences of prior *Mg* infections on reproductive health. Such data are particularly relevant for at risk populations in resource poor areas.

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aaok@ssi.dk jsj@ssi.dk