How to Collect Equine Genital Swabs in Stud Practice

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Why collect genital swabs from horses?

- Venereal disease control
  - To identify actively infected stallions and mares
  - To identify carrier stallions and mares
  - To identify carrier teasers
  - To identify contaminated semen and equipment used for artificial insemination (AI)
Why collect genital swabs from horses?

- To maintain freedom from recognised equine bacterial venereal diseases for the benefit of:
  - Mares and stallions
  - Stud farm businesses
  - Equine industries
  - National equine industries
Why collect genital swabs from horses?

• To optimise reproductive efficiency
  • Maximum fertility required at stud farms

• To satisfy industry pre-breeding requirements
  • HBLB Codes of Practice

• To satisfy sales and export requirements

• Applies to AI as well as natural cover
Venereal Disease Control

- Identification of symptomless carriers and active clinical cases of the three recognised true equine venereal bacterial pathogens:
  - *Taylorella equigenitalis* (Contagious Equine Metritis, CEMO)
  - *Klebsiella pneumoniae*
    - Capsule types 1, 2 and 5
  - *Pseudomonas aeruginosa*
Venereal Disease Control

- HBLB Codes of Practice
  - Industry standard operating procedures
  - Annual reviews
  - France, Germany, Ireland, Italy and UK
  - www.hblb.org.uk
  - http://codes.hblb.org.uk/
  - smartphone ‘app’ for 2013
Optimising Reproductive Efficiency

- Maximising conception rates
- Minimising pregnancy failures
- Maintaining the long-term health of the mare’s endometrium
  - *Streptococcus zooepidemicus*
  - *Escherichia coli*
  - *Staphylococcus aureus*
- Maintaining the health of stallions
- Semen quality control for AI
Principles of Venereal Disease Control

• Before the season starts and during the season
• Identify carrier stallions and teasers by swabbing
• Identify acutely infected or carrier mares by swabbing
• Prevent them from breeding (natural cover and AI) until treated and proven clear
• If already covered or inseminated, investigate contacts to identify extent of direct and lateral spread by swabbing
• Quality of swabbing is an essential prerequisite
Swab Sampling

- Swabs collected, certified and handled by adequately experienced veterinary surgeons, using recommended techniques and equipment

- Swabs received, cultured and results certified by HBLB quality assured (approved) laboratories (within 48 hours of collection for CEMO bacterial cultures, but not necessarily for qPCR)

- Label swabs clearly and accurately (horse name and sites swabbed) for accurate and acceptable certification on official certificates

- Always use Amies charcoal transport medium for swabs

- Pack swabs securely for transit to laboratory

- Swabs for bacterial culture (but not for CEMO qPCR) must arrive at laboratory within 48 hours of collection
Stallions and Teasers

- Take swabs after 1st January each year
- Before the stallion or teaser is used for natural mating, teasing or semen collection for AI
Two sets of swabs should be taken by a veterinary surgeon, at an interval of no less than seven days, from the stallion’s or teaser’s urethra, urethral fossa and diverticulum, penile sheath (prepuce), pre-ejaculatory fluid.

Stallion teased to penile erection, penis held with a gloved hand, gloves changed between each stallion.

Swabs examined for aerobic and microaerophilic culture and/or CEMO or multiplex (CEMO, *K. pneumoniae* and *P. aeruginosa*) qPCR at an HBLB approved laboratory.
Swab types - culture & qPCR

- Normal-tipped
  - Urethra
  - Urethral fossa and diverticulum
  - Preputial smegma
- Transport medium
  - Amies charcoal
  - All bacteriological cultures and qPCR tests
Urethral Swabs

- Pre-moisten swab in transport medium
- Swab into urethra up to the ‘hilt’
- Rotate for a few seconds
- Withdraw and place into transport medium
Urethral Fossa Swabs

- Pre-moisten swab in transport medium
- Swab into urethral fossa and diverticulum
- Rotate for a few seconds and collect smegma inc. ‘pea’ (see image), if present
- Withdraw and place into transport medium
Preputial Swabs

• Pre-moisten swab in transport medium
• Swab shaft of penis and folds of preputial reflection
• Withdraw and place into transport medium
Pre-ejaculatory fluid

- Stand back and allow teasing to proceed
- Wait until fluid drips from urethra
- Collect another urethral swab
- Withdraw and place into transport medium
Mares - clitoral swabs

- Take swabs after 1\textsuperscript{st} January each year
- Before the mare is used for natural mating or AI
- Delay swabbing for later foaling mares but don’t forget them (or repeat)
- Mares following dystocia treatments need extra swabs (see later)
Swab types

- Narrow-tipped
  - Clitoral sinus
- Normal-tipped
  - Urethral opening
  - Clitoral fossa
- Transport medium
  - Amies with charcoal for all bacterial cultures and qPCR
- No transport medium
  - Endometrial smears
Mares - clitoral swabs

- A swab or swabs should be taken by a veterinary surgeon from the mare’s
  - urethral opening
  - clitoral fossa
  - clitoral sinuses
- Mare restrained as for gynaecological examination, ideally in stocks
- Attendant wearing disposable gloves changed for each mare
- Swabs examined for aerobic and microaerophilic culture and/or CEMO or multiplex (CEMO, *K. pneumoniae* and *P. aeruginosa*) qPCR at an HBLB approved laboratory
Urethral Opening

- Wash vulva/perineum with plain water only
- Part vulval lips
- Large-tipped swab into urethral opening
- Rotate for a few seconds
- Withdraw and place into transport medium
Clitoris and Fossa - Anatomy

- At ventral commissure of vulva
- Vestibular fold
- Clitoral body
- Clitoral sinus
- Clitoral fossa
- Smegma in sinus, fossa and crevices of clitoris
Clitoral Fossa

- Wash vulva/perineum with plain water only
- Evert clitoris
- Large-tipped swab into clitoral fossa
- Swab all areas of fossa
- Withdraw and place into transport medium
Clitoral Sinus

- Wash vulva/perineum with plain water only
- Evert clitoris
- Narrow-tipped swab into central clitoral sinus
- Rotate for a few seconds
- Withdraw and place into transport medium
- Collect ‘pea’ of smegma if available
Combined urethral opening, clitoral fossa & sinus swab

- Use for industry screening only
- Do not use for export swabs
- Narrow-tipped swab in urethral opening, then rotate in central clitoral sinus and then all around clitoral fossa
- Withdraw and place into transport medium
- Collect ‘pea’ of smegma from sinus if available
Mares - endometrial swabs

- A swab or swabs should be taken by a veterinary surgeon from the mare’s endometrium during oestrus
- Submit swabs for aerobic (low-risk mares*) and microaerophilic cultures or CEMO qPCR (high-risk mares and mares with signs of inflammation or discharge or ‘short cycling’ mares*)
- Mares must be in oestrus with a relaxed cervix in order to be able to pass extended swabs through into uterus
- *Many TB stallion managers will require both aerobic and microaerophilic (or qPCR) culture results for all mares, as recommended by Newmarket Stud Farmers’ Association
Mares - endometrial smears

- Mare must be in oestrus with a relaxed cervix

- Smears are a ‘red flag’ screening test for acute endometritis
  - Endometrial smear cytology
    - Endometrial epithelial cells - smear quality control
    - Polymorphonuclear leucocytes (PMNs) - signs of inflammation (should be none or only the occasional PMN seen in smear)
    - Results may be reported as: 0 (none), +/- (occasional - <0.5% of cells seen), 1+ (few - 0.5-5% cells seen), 2+ (moderate - 5-30% of cells seen), 3+ (many - >30% cell seen)
  - Only way to accurately interpret the significance of bacteriological results in terms of association with acute endometritis
Endometrial swab types and equipment

- Normal-tipped and extended
  - Endometrial swabs
  - Endometrial smears
- Sterile single use speculum and pen light torch
- Transport medium
  - Bacteriological cultures and qPCR tests
- No transport medium
  - Cytological smears
Endometrial Swabs

- Wash vulva/perineum with plain water only
- Sterile vaginal speculum
- Extended large-tipped swab through relaxed cervix into uterus
Endometrial Swabs

- Rotate against the endometrium for a few seconds
- Withdraw and place into transport medium (swab)
- Take both swab and smear samples one after the other
Endometrial Smears

- Rotate against endometrium for a few seconds
- Withdraw and place into empty container (no transport medium)
- Make smear (gelatine-coated slides), fix and refer or use pre-stained slides (Testsimplets, Waldeck) for on-site examination
Acute endometritis bacterial isolates are opportunistic pathogens; mare is not fit for mating.

Treat mare and wait until next oestrus then re-swab/smear to confirm ‘clean’ before mating.

Normal endometrium bacterial isolates are contaminants/commensals; mare is fit for mating.

Concurrent swabs and smears:
- Normal endometrial epithelial cells only
- $\geq 1^+ \text{PMNs}$

Acute endometritis bacterial isolates are opportunistic pathogens; mare is not fit for mating.

Treat mare and wait until next oestrus then re-swab/smear to confirm ‘clean’ before mating.
For further advice

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For further information

- HBLB Codes of Practice
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  - http://codes.hblb.org.uk/
  - smartphone ‘app’ EquiBioSafe