

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?

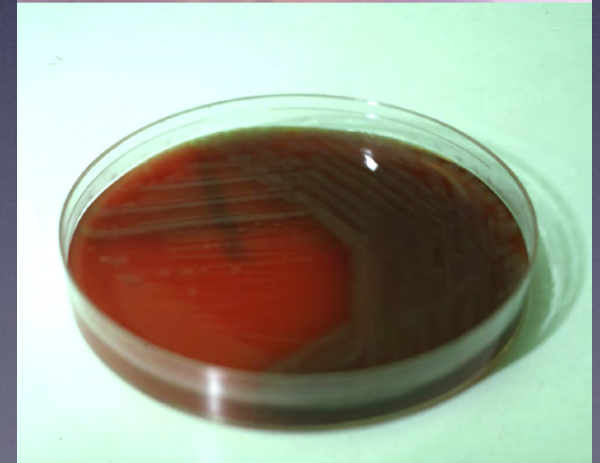
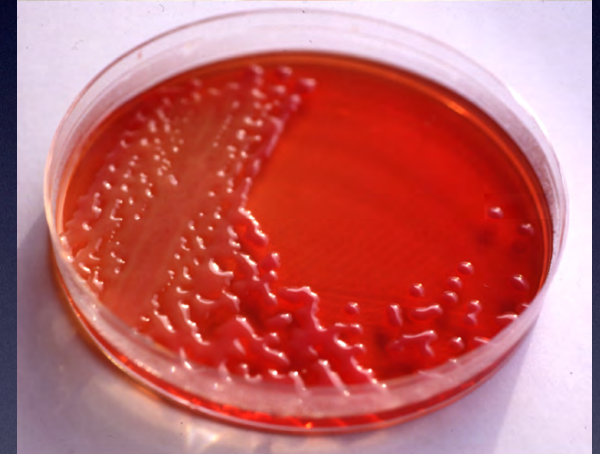
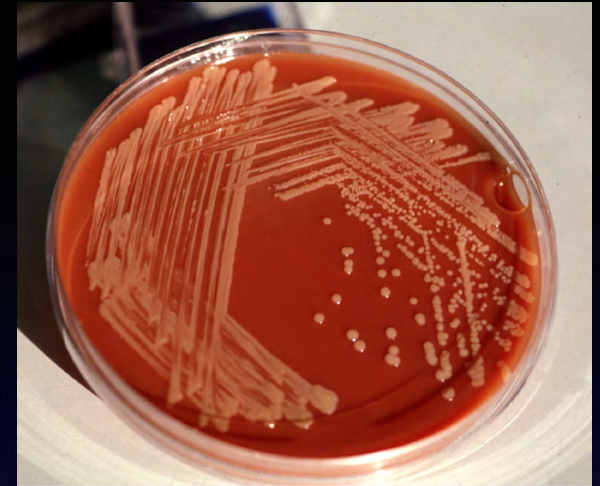
- To maintain freedom from recognised equine bacterial venereal diseases for the benefit of:
 - Mares and stallions
 - Stud farm businesses
 - Equine industries
 - National equine industries

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

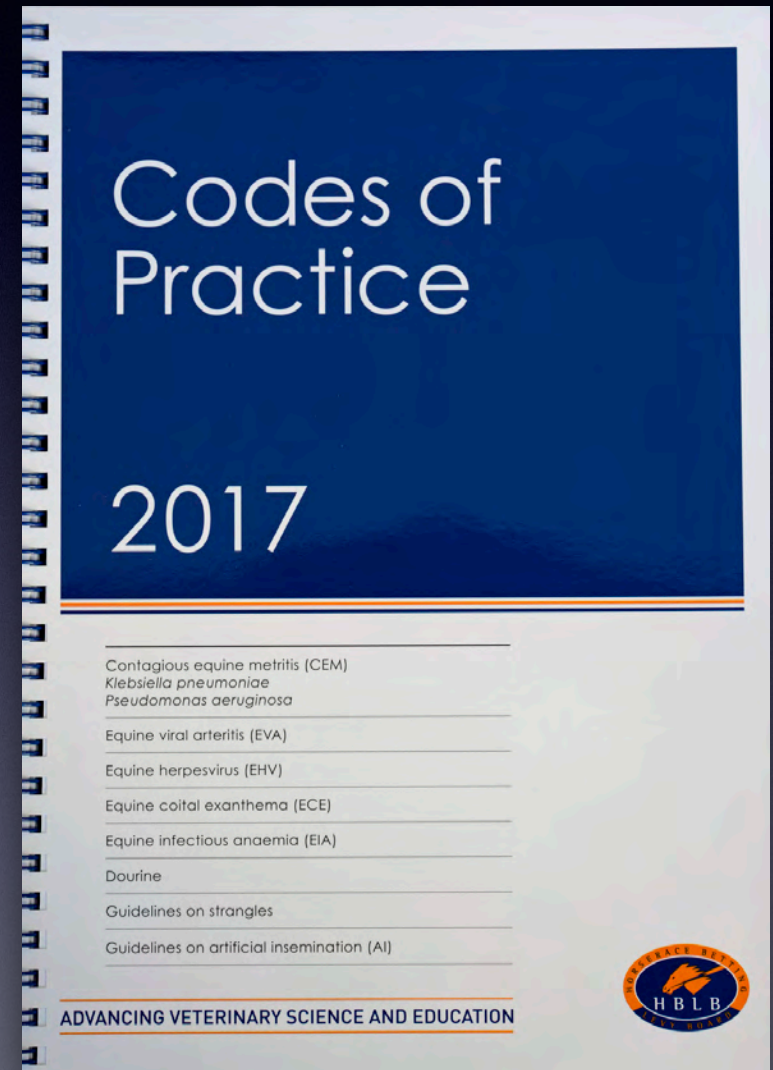
Venereal Disease Control

- 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



Stallions and Teasers

- 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 1st 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)



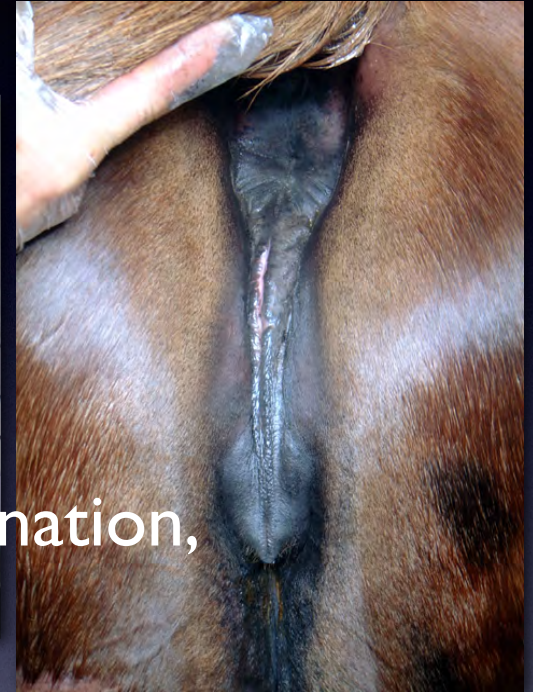
Swab types

- 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



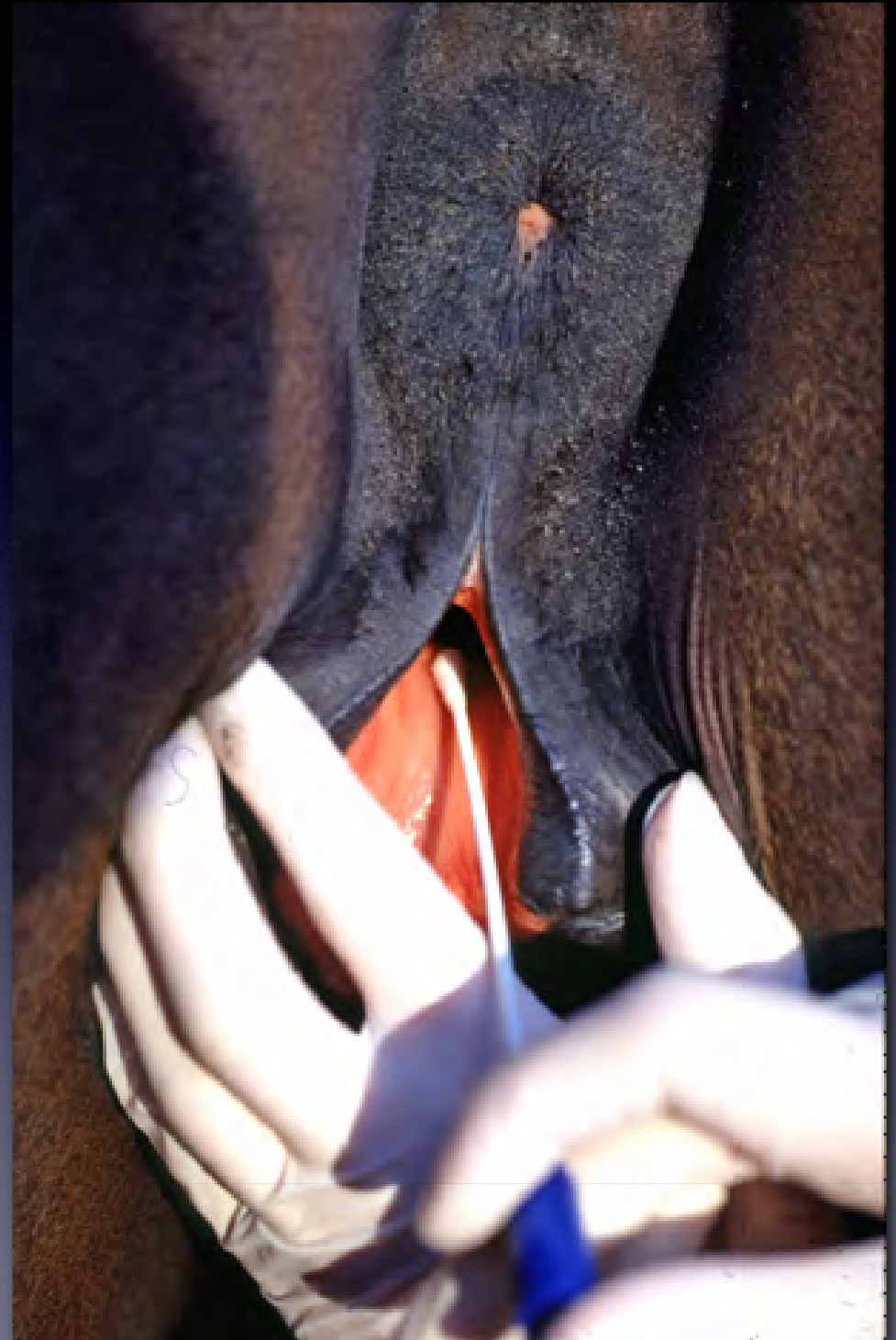
Mares - clitoral swabs

- 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



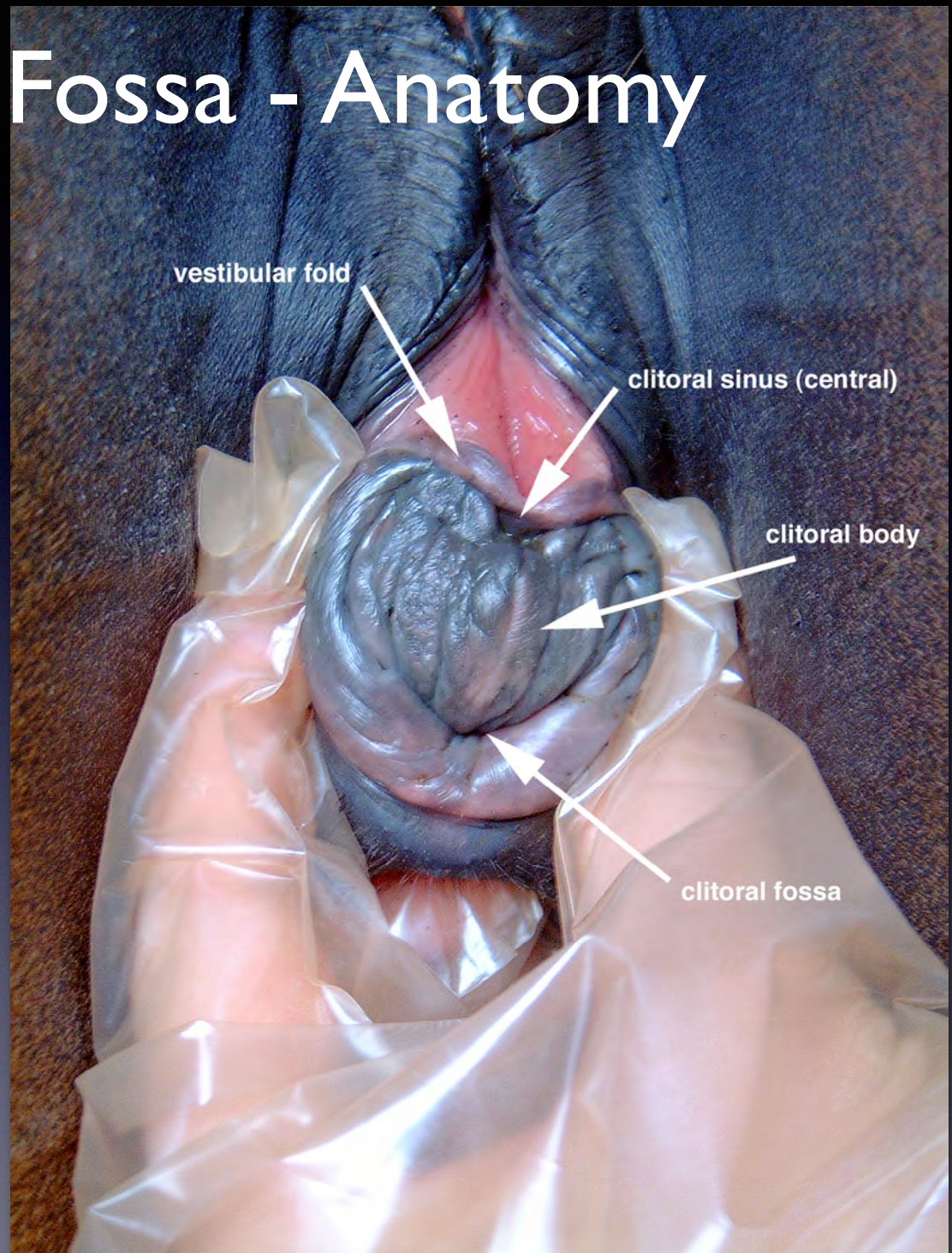
Urethral Opening

- 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



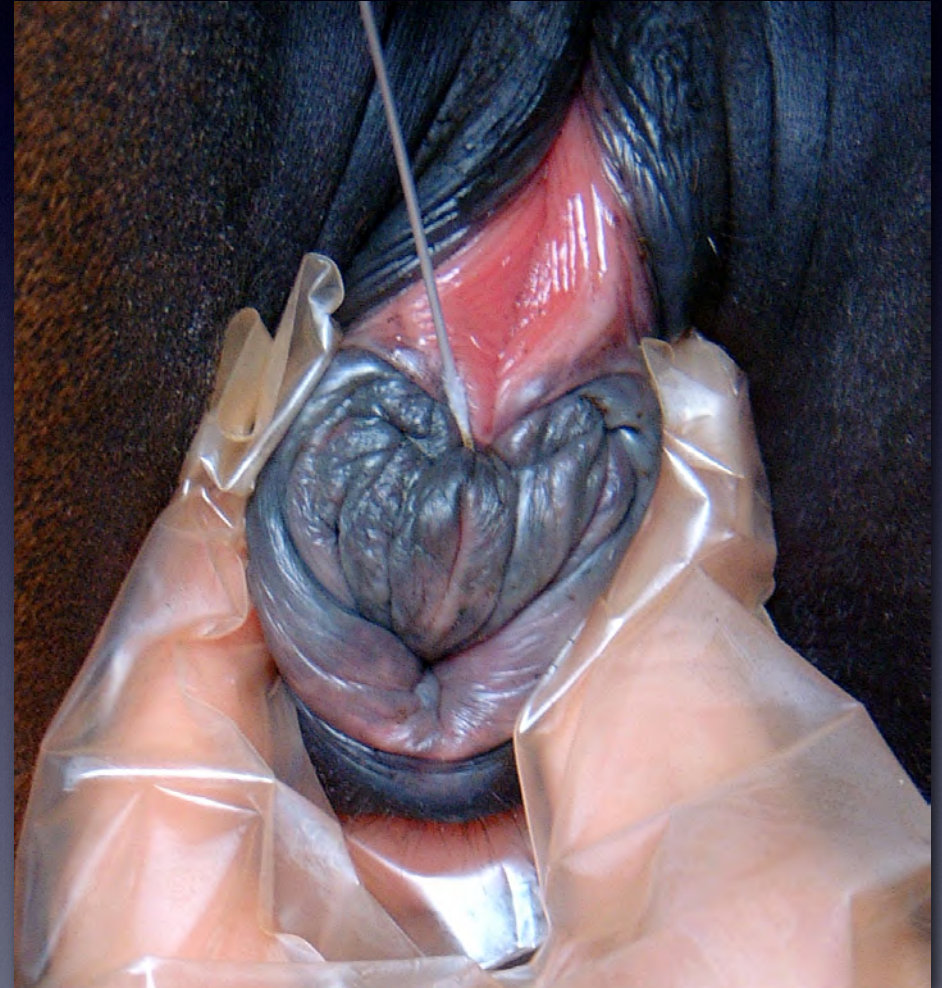
Clitoral Fossa

- 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



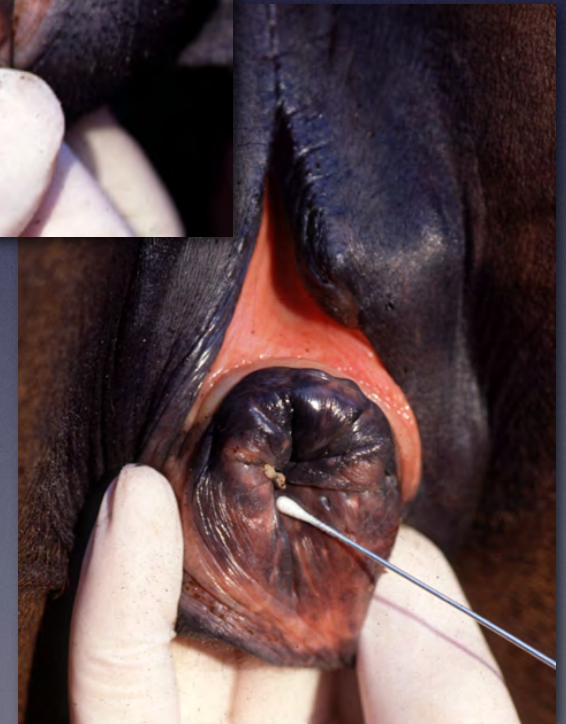
Clitoral Sinus

- 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

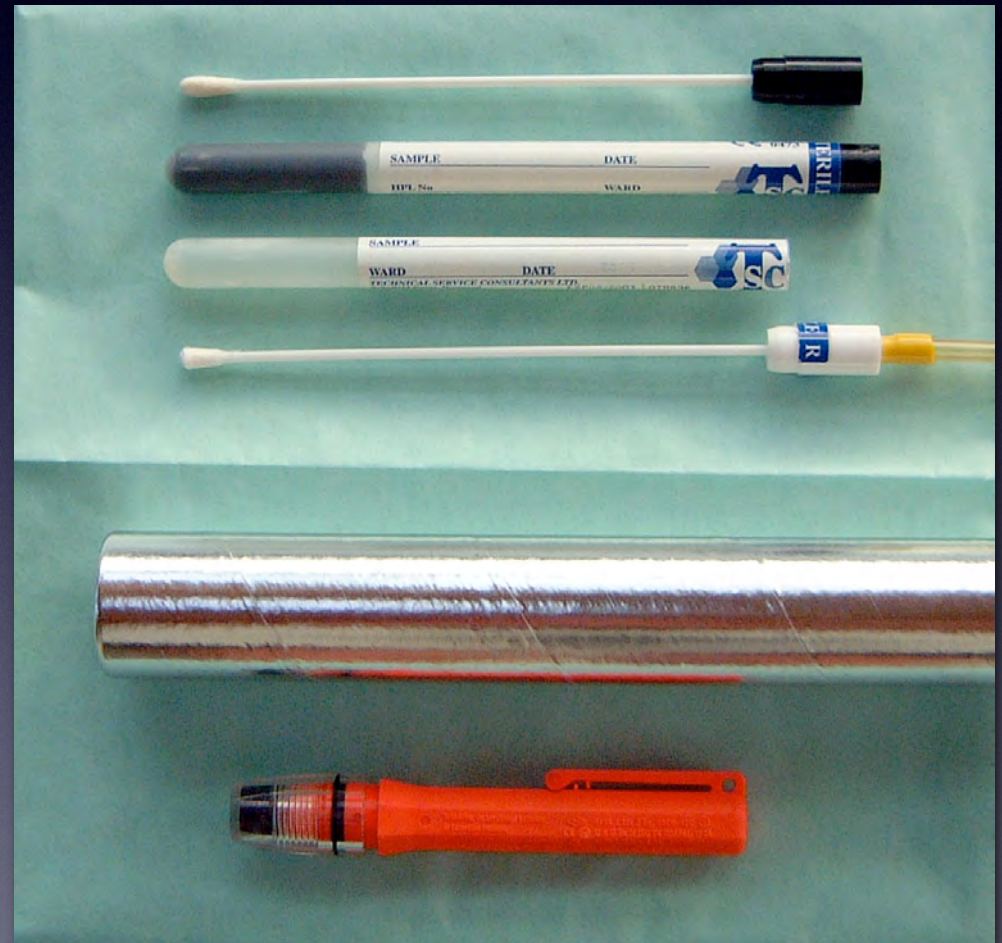
- A swab or swabs should be taken by a veterinary surgeon from the mare's endometrium during oestrus
- Submit swabs for aerobic (low-risk mares*) and microaerophilic cultures or CEMO qPCR (high-risk mares and mares with signs of inflammation or discharge or 'short cycling' mares*)
- Mares must be in 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



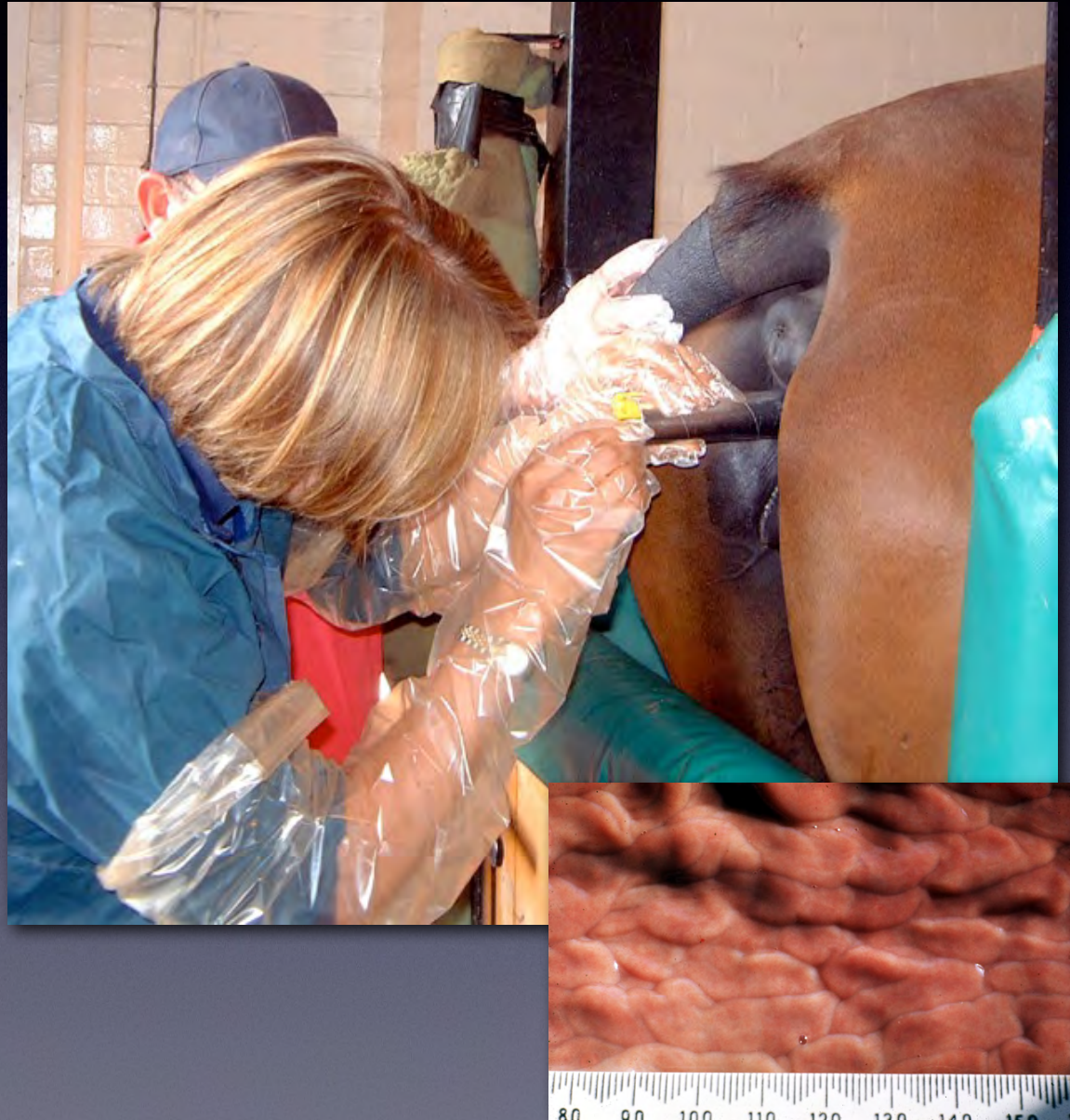
Endometrial Swabs

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



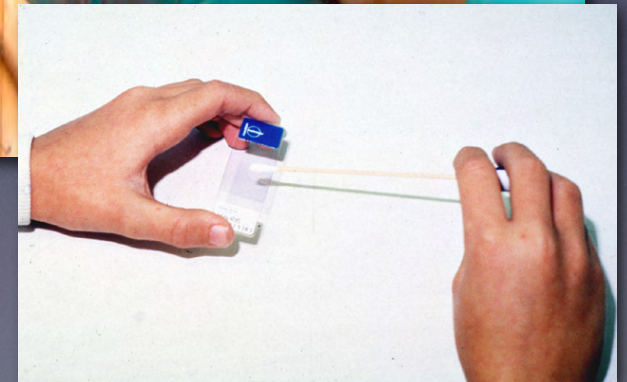
Endometrial Swabs

- 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

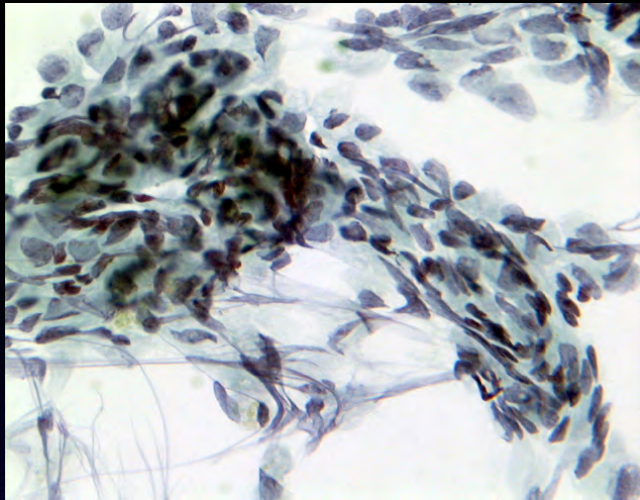


Endometrial Smears

- 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



Concurrent swabs and smears

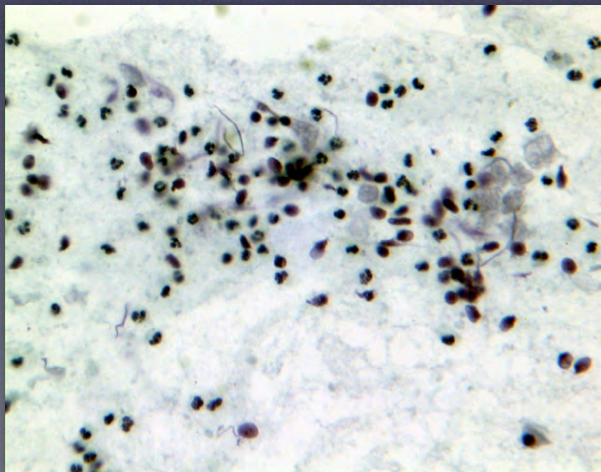


Normal endometrial epithelial cells only

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



$\geq 1+$ PMNs



Acute endometritis
bacterial isolates are opportunist 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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- Email: laboratory@rossdales.com
- Website: www.rossdales.com/laboratories

For further information

- HBLB Codes of Practice
 - www.hblb.org.uk
 - <http://codes.hblb.org.uk/>
 - smartphone 'app' EquiBioSafe

