# SPECIFICITY OF A HAND-HELD IMMUNOCHROMATOGRAPHIC ASSAY FOR ANTHRAX IN CATTLE

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## ABSTRACT

The purpose of this study was to determine whether the hand-held immunochromatographic assay was a reliable method for field based diagnosis of anthrax. This study was designed and conducted with an emphasis on obtaining an estimate of performance accuracy, in terms of specificity for the protective antigen (PA) component of the anthrax toxin. To examine specificity the hand-held assay had to be assessed under similar circumstances to that experienced in the field from typical animals in which anthrax may be suspected.

To achieve this, blood samples were collected post-mortem from 240 cattle at the Stanhope and Camperdown knackeries in Victoria. Blood smears were prepared, hand-held assays were performed on-site and a sample of blood transported back to the laboratory for bacterial culture. All 240 samples gave negative results in the hand-held assay and *B. anthracis* was not detected in any sample by culture or blood smear, which were considered the definitive diagnostic tests. Thus the hand-held assay was regarded as 100% specific (98.5-100%; 95% CI) for these cattle examined in Victoria.

The purpose of the second study was to determine whether the live Sterne strain $34F_2$ vaccine for anthrax would result in false positives arising in the hand-held assay in cattle recently vaccinated. Ten cattle were vaccinated with the $34F_2$ vaccine and monitored for 15 days. No PA was detected in the blood of vaccinated cattle in the hand-held assay or on culture within this time. These results show that the hand-held assay does not give false positive test

results in cattle post-vaccination with live $34F_2$ *B. anthracis* vaccine. The hand-held assay can be used with confidence on samples from recently vaccinated cattle that have died when it is necessary to know whether they had succumbed to anthrax or not.

The hand-held assay has the potential to be adopted as a routine test for the preliminary assessment of sudden death in cattle. The simplicity of the assay enables it to be used by unskilled lay people, which means that it could be used by knackery workers for surveillance in areas with a previous history of disease or by veterinarians as a preliminary routine tool in investigating sudden death in cattle. However the study described in this thesis only assesses the specificity of the assay and a further study, involving a similar number of cattle affected with anthrax, needs to be conducted to assess the sensitivity of the assay.

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| | |
|---|---|
| ***B. anthracis*** | *Bacillus anthracis* |
| ***B. mycoides*** | *Bacillus mycoides* |
| **cfu** | colony forming units |
| **CI** | confidence interval |
| **cm** | centimetre |
| **$CO_2$** | carbon dioxide |
| **°C** | degree celcius |
| **D+** | disease present |
| **D-** | disease absent |
| **EDTA** | ethylenediamine tetraacetic acid |
| **EF** | oedema factor |
| **ELISA** | Enzyme Linked Immunosorbent Assay |
| **ELLA** | Enzyme-Linked Lectinosorbent Assay |
| **ET** | oedema toxin |
| **FP** | false positive |
| **g** | gram |
| **h** | hour |
| **IgG** | Immunoglobulin G |
| **IHA** | indirect haemagglutination assay |
| **kDa** | kilodalton |
| **km** | kilometre |
| **LF** | lethal factor |
| **LT** | lethal toxin |

| | |
|---|---|
| **mm** | millimetre |
| **mL** | millilitre |
| **ng** | nanogram |
| **NPV** | Negative predictive value |
| **P** | pretest probability |
| **PA** | Protective antigen |
| **PCR** | Polymerase chain reaction |
| **PLET** | polymyxin-lysozyme-EDTA-thallous acetate |
| **PPV** | Positive predictive value |
| **rpm** | revolutions per minute |
| **s** | seconds |
| **SBA** | sheep blood agar |
| ***Se*** | sensitivity |
| ***Sp*** | specificity |
| **T+** | test positive |
| **T-** | test negative |
| **TN** | true negative |
| **TNF** | tumour necrosis factor |
| **TP** | true positive |
| **%** | percent |
| **μg** | microgram |
| **μm** | micrometre |