Inactivation of *Burkholderia mallei* in equine serum for laboratory use.

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The bacterium *Burkholderia mallei* is the cause of glanders. In the UK the disease was eradicated from horses and other equids in 1928 and is now rarely reported throughout the world. Freedom from disease is maintained in part by serological screening prior to international travel. Serological testing is usually by the complement fixation test (CFT) as defined in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (1).

Samples are usually submitted to laboratories as whole blood. Prior to CFT the serum is decanted and heat treated at 56-58°C for 30 minutes to inactivate the complement in the sera to avoid non-specific reactions in the CFT.

Detection of bacteraemia in glanders seems to be uncommon in even in clinical cases. (2,3,4). However concern has been raised that positive serological reactions indicate suspicion of *B.mallei* infection and hence the possibility of the organism being present in the serum which should be regarded as a potential risk to laboratory workers.

The purpose of this study was to identify if the serum inactivation procedure for CF testing also inactivates any potential *B.mallei* organisms in the blood sample.

Five to seven ml venous blood samples were taken from each of 2 healthy horses into plain vacutainers. Initially we examined the viability of the spiking method to produce ‘infected’ blood. From both samples 250µl were cultured onto Columbian blood (CBA) and nutrient agar and incubated for 24 hours at 37°C prior to being inoculated with *B.mallei*. (1 OIE). These blood samples were then seeded with 50-
100 cfu of *B. mallei* (NCTC12938) added to each whole blood and cultured onto CBA and incubated as before. The bloods were then centrifuged and the serum removed and cultured as for the whole blood. The sera were then heated at 56°C for 30 minutes in a waterbath (the heat inactivation step) and cultured as before. Both blood and serum from the two samples produced growth pre inactivation. A 1ml aliquot was taken from one of the blood samples to enumerate the number of organisms present. Colony counts prior to inactivation are shown in the table. There was a nil colony count in the serum after the inactivation step.

We then established the minimum period for heat treatment that reduced the sample to a nil viable organism count. A single fresh whole blood sample was seeded with a heavy inoculum of *B. mallei* to establish at least 10^6 cfu/ml as used in the first part of the study. The period for inactivation was estimated by removing and culturing 100µl aliquots of sera after 10, 20 and 30 minutes of heat treatment. One aliquot was cultured prior to heat treatment as a control. The remaining 3 aliquots were heat treated, one aliquot was removed at each of the three times points, starting from when the serum sample was immersed into a water bath at 56°C. From each removed aliquot 100µl was plated out for culture. All cultures were incubated for 6 days and colony counts made at 24, 48, 72 and 144 hours incubation.

In the first part of the study there was no viable growth 24 hours after heat inactivation at 56°C for 30 minutes in either of the serum samples. In the second part, viability is almost nil after only 10 minutes of heat treatment. After 20 minutes it was not possible to recover viable organisms by culture at any time point for the 6 days of incubation. The untreated control sample produced confluent growth at 24 hours.
There have been few examinations of blood samples from horses at known stages of infection with *B. mallei* (2). In non-clinically infected animals the organism is found in certain tissues at low bacterial loads (5). Culturing of blood in glandered animals has rarely recovered the organism (2, 3). This study produced high levels of viable organism in the whole blood, and represents a very sturdy challenge to the inactivation process. The centrifugation and separation process from whole blood appears to remove most of the viable organisms; the remainder are inactivated by the heat treatment.

This was a pilot study using only 1 strain of *B. mallei* in a very small number of samples. However the viable cell counts from spiking were high and the magnitude of effect of the standard complement heat inactivation step indicates this is a plausible method for reducing or negating the risk of laboratory acquired infection and may merit investigation of any variation between strains and blood samples.

Footnote
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References
1. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2014), chapter 2.5.11 Glanders

2. Lopez, J; Copps, J; Wilhelmsen, C; Moore, R; Kubay, J; St-Jacques, M; Halayko, S; Kranendonk, C; Toback, S; DeShazer, D; Fritz, DL; Tom, M; Woods, DE. (2003) Characterization of experimental equine glanders. Microbes and Infection, 5, 1125-1131


4. Howe, C; Miller, WR. (1947) Human Glanders; report of six cases. Annals of Internal Medicine, 26, 93-115.

5. Scholz, HC; Joseph, M; Tomaso, H; Al Dahouk, S; Witte, A; Kinne, J; Hagen, RM; Wernery, R; Wernery, U; Neubauer, H. (2006) Detection of the re-emerging agent
Burkholderia mallei in a recent outbreak of glanders in the United Arab Emirates by a newly developed fliP-based pCR assay. Diagnostic Microbiology and Infectious Disease 54, 241-247
Table Culture results for studies 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Pre-inoculation</th>
<th>24hrs post inoculation</th>
<th>Pre-inactivation</th>
<th>7 days post inactivation</th>
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</thead>
<tbody>
<tr>
<td><strong>Whole clotted blood</strong></td>
<td>Nil</td>
<td>Confluent growth</td>
<td>Confluent growth 1.6 x 10^6 cfu/ml</td>
<td></td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td>Light Confluent growth 1.9 x 10^5 cfu/ml</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Study 2 Serum, from spiked blood sample, inactivated for 10, 20 and 30 minutes at 56°C then incubated for 24-144 hours.

<table>
<thead>
<tr>
<th></th>
<th>Control (0 mins)</th>
<th>10mins</th>
<th>20mins</th>
<th>30mins</th>
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</thead>
<tbody>
<tr>
<td><strong>24 hours</strong></td>
<td>Confluent growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td><strong>48 hours</strong></td>
<td>Confluent growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
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<tr>
<td><strong>72 hours</strong></td>
<td>Confluent growth</td>
<td>4 colonies</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td><strong>144 hours</strong></td>
<td>Confluent growth</td>
<td>4 colonies</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
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