An Investigation of the Ability of the Glutaraldehyde Test to Distinguish between Acute and Chronic Inflammatory Disease in Horses

By P. Brink1, J.C. Wright2, and J. Schumacher3

1ATG Equine Clinic, Jägersro, 21237 Malmö, Sweden, 2Department of Pathobiology and 3Department of Clinical Sciences, Auburn University, Auburn, AL 36849-5522, USA.

Introduction

The glutaraldehyde reagent in the glutaraldehyde test (GT) creates a clot with either fibrinogen or gammaglobulin in EDTA-stabilized blood by chemical reaction between the aldehyde groups in glutaraldehyde and free amino groups in fibrinogen and immunoglobulins (Sandholm 1974a, Sandholm 1974b, Martin et al. 1985). The process is believed to run as a first degree chemical reaction, where the reaction time is directly proportional to the concentration of fibrinogen and immunoglobulins (Sandholm 1974a, Sandholm 1974b, Eriksen 1984).

The rapid and inexpensive GT has been used with success empirically in Europe for many years for diagnosing inflammatory diseases in cattle (Sandholm 1974a, Sandholm 1974b, Liberg et al. 1975a, Liberg et al. 1975b, Nielsen 1985).
1975, Martens 1977, Liberg 1978, Tennant et al. 1979, Liberg 1981, Liberg 1982, Eriksen 1984, Keulen et al. 1984, Doll et al. 1985, Larsson 1985, Mahlin et al. 1985, Chadli & Mahin 1986, Kovac 1988, Katholm & Jorgensen 1992, Kantor et al. 1993, Tyler et al. 1996, Sen et al. 2000, Ramprabhu et al. 2002), pigs (Liberg 1979, Hansen 1985, Kovac et al. 1993), goats (Satpathy et al. 1996, Vihan 1989), mink (Sandholm & Kangas 1973), dogs (Sandholm & Kivisto 1975, Wolff 1986), and zoo animals (O’Rourke & Satterfield 1981, Carstairs-Grant et al. 1988, Juyal & Uppal 1995). In these species, the test was used to indicate whether an inflammatory disease was acute or chronic (Doll et al. 1985, Chadli & Mahin 1986).

The GT, because of its simplicity, is very useful in bovine practice for rapidly diagnosing inflammation under circumstances where it is not practical or economically possible to have blood analyzed at a professional clinical laboratory (Sandholm 1974a, Sandholm 1974b, Liberg et al. 1975a, Liberg et al. 1975b, Nielsen 1975, Martens 1977, Liberg 1978, Tennant et al. 1979, Liberg 1981, Liberg 1982, Eriksen 1984, Keulen et al. 1984, Doll et al. 1985, Larsson 1985, Mahlin et al. 1985, Chadli & Mahin 1986, Kovac 1988, Katholm & Jorgensen 1992, Kantor et al. 1993, Tyler et al. 1996, Sen et al. 2000, Ramprabhu et al. 2002).

A negative GT can be used as a semiquantitative indicator of hypogammaglobulinemia caused by failure of passive transfer of colostrum in neonatal foals (Beetson et al. 1985, Clabough et al. 1989, Saikku et al. 1989, Clabough et al. 1991, Kumaran & Bhuvanakumar 1994, Kalinbacak & Or 1996, Brujin et al. 2003), calves (Tennant et al. 1979, Keulen et al. 1984, Larsson 1985, Kovac 1988, Tyler et al. 1996, Sen et al. 2000), kids (Vihan 1989, Satpathy et al. 1996), and zoo ruminants (O’Rourke & Satterfield 1981, Carstairs-Grant et al. 1988, Juyal & Uppal 1995). The GT also has been used to determine the content of IgG in mare colostrum (Jones & Brook 1995, Ezhilan & Bhuvanakumar 1998).

Clinical experience indicates that the GT may not be as reliable in horses as it is in cattle (Nielsen 1975). In horses, lack of reliability of the GT has been proposed to be caused by generally lower or delayed peaks of concentrations of fibrinogen and immunoglobulin or a different distribution of immunoglobulins (IgG, IgM, IgA) compared to cattle (Bendixen 1954, Nansen & Nielsen 1966, Sandholm 1974a, Nielsen 1975, Aasted et al. 1989).

The purpose of this clinical trial was to determine the ability of GT to distinguish between acute and chronic inflammatory disease in horses. During the trial we compared indicators of inflammation (the concentration of blood fibrinogen and serum globulin) to the GT.

Materials and methods
Thirty seven horses admitted for investigation of suspected inflammatory disease were evaluated using the GT (Glutarvaca®), a complete clinical examination, CBC and routine serum biochemistries that included total protein, albumin, globulin and fibrinogen. Blood for the GT and laboratory analysis was collected at the same time either upon arrival at the hospital or the following day.

Horses having a history of clinical signs of inflammatory disease of total duration six days or less were arbitrarily classified as acutely inflamed. Horses with a history of clinical signs greater than six days were arbitrarily classified as chronically inflamed. The clinical examination leading to the diagnosis and etiology was also used to reinforce the distinction between acute and chronic disease (Table 1).

The GT was performed by adding equal amounts of fresh blood and glutaraldehyde in a test tube, mixing by slowly turning the test tube and visually observing and noting the time re-
Table 1: Diagnosis and outcome.

| Horse # | Diagnosis                                                                 | Duration  | Outcome                      |
|---------|---------------------------------------------------------------------------|-----------|------------------------------|
| 1       | Purulent, bilateral guttural pouch empyema                                  | Chronic   | Fatal (spontaneous)          |
| 2       | Dorsal rectal abscess                                                      | Chronic   | Discharged                   |
| 3       | Traumatic, infected joint capsular laceration                              | Acute     | Discharged                   |
| 4       | Dorsal rectal abscesses                                                   | Chronic   | Discharged                   |
| 5       | Purulent nephritis, lung abscesses, ulcerous dermatitis, myocarditis, fatty liver | Chronic   | Fatal (euthanasia)           |
| 6       | Severe, idiopathic, systemic infection                                     | Acute     | Fatal (spontaneous)          |
| 7       | Purulent (jugular) thrombophlebitis (abscess)                              | Chronic   | Discharged                   |
| 8       | Transportation syndrome, bronchitis/pleuritis, systemic infection          | Acute     | Discharged                   |
| 9       | Fibrinopurulent pleuropneumonia                                            | Acute     | Fatal (euthanasia)           |
| 10      | Systemic, malign lymphoma, borrelia infection                              | Chronic   | Fatal (euthanasia)           |
| 11      | Infected tendovaginitis                                                   | Acute     | Discharged                   |
| 12      | Intraabdominal abscess, squamous cell carcinoma (ventricle)               | Chronic   | Fatal (euthanasia)           |
| 13      | Septic, purulent arthritis                                                 | Chronic   | Discharged                   |
| 14      | Fibrinopurulent pleuropneumonia                                            | Acute     | Fatal (euthanasia)           |
| 15      | Septicemia, pneumonia, peritonitis                                         | Acute     | Fatal (euthanasia)           |
| 16      | Severe, purulent, traumatic muscle laceration                              | Chronic   | Discharged                   |
| 17      | Severe, iatrogenic, muscle abscesses                                       | Chronic   | Discharged                   |
| 18      | Purulent osteomyelitis                                                    | Chronic   | Fatal (euthanasia)           |
| 19      | Severe subcutaneous infection/abscess, funiculitis                         | Chronic   | Discharged                   |
| 20      | Humerus fracture, subcutaneous infection/abscess                           | Chronic   | Discharged                   |
| 21      | Bacterial diarrhea                                                         | Acute     | Fatal (euthanasia)           |
| 22      | Abscess, inguinal region                                                   | Chronic   | Discharged                   |
| 23      | Scrotal abscesses, postoperative castration                                | Chronic   | Discharged                   |
| 24      | Necrotizing myositis, multiple subcutaneous abscesses                      | Chronic   | Discharged                   |
| 25      | Fibrinopurulent septic bicepital bursitis, muscular septic cellulitis      | Chronic   | Fatal (euthanasia)           |
| 26      | Pericarditis, mitral insufficiency, systemic infection                     | Chronic   | Fatal (euthanasia)           |
| 27      | Septic peritonitis                                                        | Chronic   | Discharged                   |
| 28      | Septic meningitis                                                         | Acute     | Discharged                   |
| 29      | Septicemia, premature foal                                                | Acute     | Discharged                   |
| 30      | M. Masseter, throat latch, parotid, jugular abscesses/fistulae             | Chronic   | Discharged                   |
| 31      | Systemic infection, septic myositis                                       | Chronic   | Fatal (euthanasia)           |
| 32      | Systemic infection, possible abdominal/kidney abscess, emaciation          | Chronic   | Fatal (euthanasia)           |
| 33      | Severe, multiple, purulent, septic arthritis                               | Chronic   | Fatal (euthanasia)           |
| 34      | Metritis, purulent peritonitis, abdominal abscesses, adherences            | Chronic   | Fatal (euthanasia)           |
| 35      | Purulent, pharyngeal inflammation, choke                                   | Acute     | Discharged                   |
| 36      | Thrombosis pulmonary vessels, Cushing disease, laminitis                  | Chronic   | Fatal (euthanasia)           |
| 37      | Systemic intoxication, parasitic aneurysm, intestinal volvulus, paralysis  | Acute     | Fatal (euthanasia)           |
Table 2: Categorization of GT-time.

| Group # | GT-time        | Empiric categorization                                      |
|---------|----------------|-------------------------------------------------------------|
| 1       | 0 < GT-time < 3 min. | High increase in concentration of fibrinogen and/or immunoglobulin |
| 2       | 3 < GT-time < 6 min. | Moderate increase in concentration of fibrinogen and/or immunoglobulin |
| 3       | 6 < GT-time < 15 min. | Low increase in concentration of fibrinogen and/or immunoglobulin |
| 4       | GT > 15 min.     | No increase in concentration of fibrinogen and/or immunoglobulin |

Table 3: Blood values

| Horse # | GT-time | Albumin (g/l) | Globulin (g/l) | Alb/ Glob (ratio) | Fibrinogen (g/l) | Total prot. (g/l) | WBC (10^9/l) | Differential cell count leukocytes |
|---------|---------|--------------|---------------|------------------|-----------------|-------------------|--------------|-----------------------------------|
|         |         |              |               |                  |                 |                   |              | Bands (%) Segm (%) Eosin (%) Mono (%) Lymph (%) Baso (%) RBC (10^12/l) Hemo- globin (g/l) PCV (%) |
| 1       | 2,0     | 30           | 58            | 0,5              | 7,1             | 88                | 9,4          | 1 63 0 4 32 0 8,8 113 31          |
| 2       | NR      | 30           | 35            | 0,9              | 7,5             | 65                | 9,3          | 0 42 0 5 53 0 9,7 118 33          |
| 3       | NR      | 38           | 30            | 1,3              | 3,6             | 68                | 9,4          | 0 67 1 1 31 0 8,5 123 34          |
| 4       | NR      | 27           | 35            | 0,8              | 4,7             | 62                | 7,0          | 3 24 2 3 66 2 8,2 110 30          |
| 5       | NR      | 41           | 33            | 1,2              | 2,3             | 74                | 16,7         | 0 85 0 5 9 1 11,6 178 48          |
| 6       | NR      | 31           | 40            | 0,8              | 8,3             | 71                | 10,2         | 2 79 0 4 15 0 7,3 117 33          |
| 7       | NR      | 36           | 37            | 1,0              | 7,7             | 73                | 16,0         | 5 78 0 1 16 0 11,7 186 50          |
| 8       | 3,5     | 34           | 42            | 0,8              | 4,4             | 76                | 9,3          | 2 68 1 3 25 1 7,3 125 35          |
| 9       | NR      | 32           | 37            | 0,9              | 9,9             | 69                | 9,2          | 11 64 0 4 21 0 8,6 144 39          |
| 10      | 1,0     | 17           | 63            | 0,3              | 3,0             | 80                | 15,8         | 0 80 1 4 14 1 1,1 27 8           |
| 11      | 31      | 48           | 12            | 1,2              | 5,9             | 63                | 11,1         | 0 68 0 3 29 0 8,0 105 31          |
| 12      | 6,0     | 32           | 60            | 0,5              | 5,2             | 92                | 7,0          | 0 74 1 5 20 0 7,0 130 34          |
| 13      | NR      | 34           | 29            | 0,6              | 8,2             | 76                | 9,3          | 2 68 1 3 25 1 7,3 125 35          |
| 14      | 5,0     | 18           | 47            | 0,4              | 7,1             | 65                | 10,3         | 3 59 0 8 30 0 9,2 153 45          |
| 15      | 23      | 27           | 9,0           | 6,4              | 50              | 2,8               | 7,1          | 7 11 0 5 77 0 12,8 176 50          |
| 16      | 25      | 35           | 1,3           | 2,9              | 58              | 7,4               | 1 68 6 1 23 1 7,8 136 38          |
| 17      | 31      | 29           | 1,1           | 5,4              | 60              | 7,3               | 0 56 2 6 36 0 10,1 168 48          |
| 18      | NR      | 27           | 25            | 1,1              | 4,9             | 52                | 35,4         | 0 94 0 3 3 0 6,5 137 39          |
| 19      | 3,0     | 29           | 58            | 0,5              | 6,8             | 87                | 19,2         | 1 72 2 1 24 0 5,8 85 26           |
| 20      | 33      | 20           | 1,7           | 12,2             | 53              | 15,0              | 0 75 0 6 19 0 9,1 121 35          |
| 21      | NR      | 20           | 1,8           | 7,0              | 38              | 36,6              | 0 90 0 1 9 0 10,3 136 37          |
| 22      | 3,0     | 21           | 69            | 0,3              | 5,0             | 90                | 30,7         | 0 85 1 1 13 0 6,3 92 24          |
| 23      | NR      | 21           | 36            | 0,6              | 6,0             | 57                | 7,5          | 0 54 2 2 41 1 6,3 107 28          |
| 24      | NR      | 21           | 1,1           | 5,0              | 40              | 13,7              | 1 87 0 2 10 0 5,5 72 19           |
| 25      | NR      | 36           | 31            | 1,2              | 6,6             | 67                | 8,6          | 0 77 0 8 14 0 5,6 101 27          |
| 26      | NR      | 35           | 22            | 1,6              | 6,0             | 57                | 10,8         | 0 86 2 2 10 0 8,3 141 41          |
| 27      | 5,0     | 31           | 46            | 0,7              | 9,0             | 77                | 13,5         | 0 72 0 3 25 0 7,4 119 32          |
| 28      | NR      | 25           | 41            | 0,6              | 10,0            | 66                | 33,4         | 0 93 0 6 1 0 10,5 126 32          |
| 29      | NR      | 32           | 16            | 2,0              | 5,0             | 48                | 0,8          | 0 16 0 0 84 0 7,7 117 31          |
| 30      | NR      | 28           | 21            | 1,3              | 14,7            | 49                | 26,4         | 0 72 0 9 18 1 8,1 98 25           |
| 31      | 15,0    | 27           | 1,5           | 7,3              | 67              | 11,5              | 1 78 1 2 17 1 6,8 118 31          |
| 32      | NR      | 36           | 19            | 1,9              | 4,6             | 55                | 15,0         | 2 42 2 7 47 0 9,3 116 32          |
| 33      | 15,0    | 29           | 44            | 0,7              | 4,8             | 73                | 8,0          | 1 45 0 9 45 0 8,0 128 35          |
| 34      | 14,0    | 21           | 23            | 0,9              | 5,8             | 44                | 9,4          | 0 90 2 2 6 0 6,2 114 31          |
| 35      | 2,0     | 31           | 42            | 0,7              | 11,0            | 73                | 12,0         | 0 75 1 2 21 1 5,1 83 20           |
| 36      | 3,0     | 32           | 41            | 0,8              | 5,0             | 73                | 16,7         | 0 93 0 2 5 0 4,3 80 20           |
| 37      | NR      | 33           | 39            | 0,8              | 6,3             | 72                | 11,9         | 0 76 0 7 17 0 11,7 185 53          |

* NR = no reaction
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Table 4: GT result versus mean blood values (+/- standard deviation).

|                | Albumin (g/l) | Globulin (g/l) | Alb/Glo (ratio) | Fibrinogen (g/l) |
|----------------|---------------|----------------|-----------------|------------------|
| GT-positive    | 27,9 (+/- 6,6) | 47,5 (+/- 13,7) | 0,7 (+/- 0,3)   | 6,6 (+/- 2,1)    |
| GT-negative    | 30,7 (+/- 5,7) | 29,3 (+/- 8,7)  | 1,1 (+/- 0,4)   | 6,6 (+/- 2,9)    |
| All horses     | 29,7 (+/- 6,1) | 36,0 (+/- 13,6) | 1,0 (+/- 0,4)   | 6,6 (+/- 2,6)    |

Table 5: Clinical parameters versus mean blood values.

|                | GT-positive (%) | Albumin (g/l) | Globulin (g/l) | Alb/Glo (ratio) | Fibrinogen (g/l) |
|----------------|-----------------|---------------|----------------|-----------------|------------------|
| Duration       |                 |               |                |                 |                  |
| Acute          | 23,1            | 26,7          | 43,0           | 0,6             | 8,8              |
| Chronic        | 76,9            | 28,2          | 48,9           | 0,7             | 5,9              |
| Outcome        |                 |               |                |                 |                  |
| Fatal          | 69,2            | 27,8          | 44,8           | 0,7             | 6,0              |
| Discharged     | 30,8            | 28,0          | 53,8           | 0,6             | 8,0              |

Table 6: GT-time groups versus mean blood values within groups (+/- standard deviation).

| Group #        | GT-positive (No) | Mean Globulin (g/l) | Mean Alb/Glo (ratio) | Mean Fibrinogen (g/l) |
|----------------|------------------|---------------------|----------------------|-----------------------|
| 1: (0<GT-time<3 min.) | 6               | 55,2 (+/- 11,3)   | 0,5 (+/- 0,2)       | 6,3 (+/- 2,7)         |
| 2: (3<GT-time<6 min.) | 4               | 48,3 (+/- 8,4)    | 0,6 (+/- 0,2)       | 7,4 (+/- 1,7)         |
| 3: (6<GT-time<15 min.) | 3               | 31,3 (+/- 11,2)   | 1,0 (+/- 0,4)       | 6,0 (+/- 2,0)         |
| 4: (GT-time>15 min.) | 24              | 29,3 (+/- 8,7)    | 1,1 (+/- 0,4)       | 6,6 (+/- 2,9)         |

Results

In Table 1, diagnoses, estimated duration of the diseases and outcome of the clinical cases are summarized.

In Table 3, the GT-times and results of blood analysis of the horses are summarized.

Table 4 shows the mean concentration of selected blood values for horses whose blood had positive reaction to the GT, compared to horses whose blood had a negative reaction to the GT. Table 5 shows the comparison of selected clinical parameters and mean blood values of horses with positive GT.

The GT-times were divided into groups as listed in Table 6. Table 7 shows the correlation of GT-time and Group number versus globulin concentration and albumin/globulin ratio, respectively, by linear regression. The regression equations are also shown in Graphs 1-4. Group number did not correlate with the mean fibrinogen concentration within groups.

Among the hospitalized horses, there was a higher fatality rate in the GT positive horses (69% = 9/13) when compared to the GT negative horses (38% = 9/24); however, this finding was not statistically significant (p=0.06, Chi square test).
Among the 37 horses, the proportion of test negatives of horses that were acutely inflamed was 75% (9/12). The proportion of acutely inflamed test negatives was significantly greater than the proportion of chronically inflamed test positives (p=0.04, Chi square test). The proportion of test positives of horses that were chronically inflamed was 40% (10/25).

The GT did not show statistically significant correlation with the concentration of blood fibrinogen in acute or chronic diseases. All results from the blood analyses (Table 3) were also compared to the GT using principal component analysis without finding any statistically significant correlation.

**Discussion**

The results of this study indicate that the GT can be used to quickly differentiate chronic from acute inflammatory disease in horses. The high proportion of test negatives of horses having acute inflammation indicates that horses with inflammatory disease and negative GT are likely to be acutely, rather than chronically, inflamed. Among GT positive horses, 77% were chronically inflamed as shown in Table 5. The GT was not reliable in predicting the blood concentration of fibrinogen in acute or chronic inflammatory diseases.

Useful clinical information could be obtained by dividing GT-times into categories (groups) as listed in Table 2 (Liberg et al. 1975a, Liberg et al. 1975b). Comparison of category and respectively globulin concentration and albumin/globulin ratio within a category seemed to correlate, although this tendency was not statistically significant. This could be due to the small number of data points. A larger number of horses included in a future study like ours would probably eliminate this statistical uncer-
The correlation above has been observed in cattle (Sandholm 1974a, Liberg et al. 1975a, Liberg et al. 1975b, Nielsen 1975, Eriksen 1984). The difference between other studies of other species and this study was that horses in Group 1 had only moderately increased globulin concentration and moderately decreased albumin/globulin ratio, Group 2 horses had a mildly increased globulin concentration and mildly decreased albumin/globulin ratio, and horses in Group 3 had a globulin concentration and albumin/globulin ratio within normal range.

If the clinical examination indicates systemic infection (eg. increased rectal temperature) and the GT is positive, the probability is high (77% likelihood) for chronic inflammatory disease. A positive GT acts then as an indicator for further laboratory analysis of blood to determine chronicity and etiology of the disease. If the test is negative, the disease is most likely acute or the systemic inflammatory response is either insignificant or absent. The GT can also be used as an additional diagnostic test to indicate prognosis because a positive test predicted fatal outcome in 69% of the clinical cases we studied. The test performance regarding the predictability of a fatal outcome might increase if only severe inflammatory diseases are included as compared to a study also including mild cases (selection bias). Also, the lack of controls will add bias to the percentages and will eliminate false positives. Because the study did not include a group of controls and a group of

Graph 2. GT-time/A/G ratio regression.

Graph 3. Regression of GT time Group vs Mean Globulin.

Graph 4. Regression of GT-time Group vs Mean Albumin/Globulin Ratio.
horses suffering from non-inflammatory diseases, the data presented can only be considered valid for horses with inflammatory disease. For this reason, the conclusions are not valid for the entire population of horses. The selection of horses among patients submitted to a large referral hospital also might introduce spectrum bias as the hospitalized horses are more likely to be severely affected than horses treated in practice.

A positive GT in horses indicated the probability of increased serum concentration of globulin and a decreased albumin/globulin ratio, but the GT was not correlated with the blood concentration of fibrinogen.

Taking into consideration the low cost and rapid application of the GT and correlation of a positive test with increased concentration of globulin, the GT is a useful screening test for horses suspected to suffer from inflammatory disease.

a) Glutarvac Test tube; Jorgen Kruuse A/S, Marslev, Denmark.

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Sammendrag
Glutaraldehydprøvens evne til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest.

Glutaraldehydprøven (GP), en hurtig og billig test, har været anvendt empirisk gennem mange år i kvægpraksis for diagnosticering af inflammatoriske sygdomme. GP bliver primært brugt til at påvise øget serum koncentration af fibrinogen og globulin. Glutaraldehyd bindes til frie amino-grupper i fibrinogen og globulin, som derpå danner et blodkoagel ved en 1. grads kemisk reaktion. Koaguleringstiden af GP estimerer indholdet af de proteiner, som produceres i et inflammatorisk respons. Anvendeligheden af GP til diagnosticering af inflammatoriske tilstande i hestepraksis har aldrig været undersøgt før. Formålet med dette studie er at bestemme GPs evne til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest. 37 heste, mistænkt for inflammatorisk sygdom, blev evalueret på basis af GP, anamnese, fuldstændig klinisk undersøgelse samt rutinemæssig blodprøve. GP-tid, blodprøvesvar og klinisk udfald blev sammenlignet statistisk. De heste, som var bestemt til at være akut afficeret på basis af anamnese, klinisk undersøgelse og rutinemæssig blodprøve, tenderede mod at have negativ GP (75%). Der kunne ikke påvises sammenhæng mellem GP og fibrinogen koncentration i blodet. Positiv GP forudsagde også et fatalt udfald i 69% af de kliniske tilfælde. Resultaterne af dette studie indikerer, at GP kan være en brugbar praktisk test til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest.

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Reprints may be obtained from: Palle Brink, ATG Equine Clinic, Jägersro, 21237 Malmö, Sweden.

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