Evaluation of serum galactomannan detection for diagnosis of feline upper respiratory tract aspergillosis.

J. Whitney\textsuperscript{a, *}, J.A. Beatty\textsuperscript{a}, P. Martin\textsuperscript{b}, N.K. Dhand\textsuperscript{c}, K. Briscoe\textsuperscript{a} and V.R. Barrs\textsuperscript{a}

\textsuperscript{a} Valentine Charlton Cat Centre, Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia.

\textsuperscript{b} Veterinary Pathology Diagnostic Services, Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia.

\textsuperscript{c} Farm Animal and Veterinary Public Health, Faculty of Veterinary Science, C01-JL Shute, University of Sydney, NSW 2570, Australia

* Corresponding author. Tel: +61 2 93513437; fax +61 2 93517436

Email address: joanna.whitney@sydney.edu.au

Keywords: feline, aspergillosis, galactomannan
Abstract
Measurement of serum galactomannan (GM), a polysaccharide fungal cell-wall component, is a non-invasive test for early diagnosis of invasive aspergillosis in humans. Feline upper respiratory tract (URT) aspergillosis is an emerging infectious disease in cats. Diagnosis requires biopsy for procurement of tissue specimens for cytological or histological detection of fungal hyphae and for fungal culture. The aim of this study was to evaluate serum GM measurement as a non-invasive diagnostic test for URT aspergillosis in cats. A one-stage, immunoenzymatic sandwich ELISA was used to detect serum GM in 4 groups of cats; Group 1 (URT aspergillosis) – confirmed URT aspergillosis (n=13, sinonasal aspergillosis (SNA) n=6, sino-orbital aspergillosis (SOA) n=7), Group 2 (URT other) – other URT diseases (n=15), Group 3 (β-lactam) – cats treated with β-lactam antibiotics for non-respiratory tract disease (n=14), Group 4a – healthy young cats (≤ 1 y of age, n=28), Group 4b – healthy adult cats (>1 y of age, n=16). One cat with SNA and two cats with SOA caused by an Aspergillus fumigatus-mimetic species, tested positive for serum GM. For a cut-off optical density index of 1.5, the overall sensitivity and specificity of the assay was 23% and 78% respectively. False positive results occurred in 29 % of cats in Group 3 and 32% of cats in Group 4a. Specificity increased to 90% when Groups 3 and 4a were excluded from the analysis. Overall, serum GM measurement has a poor sensitivity but is a moderately specific, non-invasive screening test to rule out infection in patients with suspected feline upper respiratory tract aspergillosis.
Introduction

Feline upper respiratory tract (URT) aspergillosis has been increasingly recognised since it was first described by Wilkinson et al., in 1982, with over 50 cases reported across three continents. (Barachetti et al., 2009, Barrs et al., 2012a, Furrow & Groman 2009, Giordano et al., 2010, Goodall et al., 1984, Hamilton et al., 2000, Kano et al., 2008, Karnik et al., 2009, McLellan et al., 2006, Smith & Hoffman, 2010, Tomsa et al., 2003, Whitney et al., 2005, Wilkinson et al., 1982). Similar to canine sinonasal aspergillosis (SNA), infections caused by *A. fumigatus* are confined to the sinonasal cavity. However, other *Aspergillus fumigatus* complex members cause more invasive disease that extends into the orbit and adjacent tissues in two-thirds of feline cases (Barrs et al., 2012a). Treatment of sino-orbital aspergillosis (SOA) is rarely successful and early initiation of therapy is precluded by failure to make a diagnosis until advanced-stage disease.

Galactomannan (GM), a water-soluble, heat-stable polysaccharide cell wall component of *Aspergillus* species is released into the circulation during hyphal invasion into tissue (Hope et al., 2005). Serum GM measurement is the most frequently used test for early diagnosis of invasive aspergillosis (IA) in at-risk human populations, although false positive results have been identified in young patients and those being treated with β-lactam antibiotics (Siemann et al., 1998, Zandijk et al., 2008). Serum GM detection is highly sensitive for diagnosis of systemic aspergillosis in dogs but has poor sensitivity and specificity for diagnosis of SNA in dogs (Billen et al., 2009, Garcia et al., 2012). In canine SNA fungal hyphae colonise sinonasal surface epithelium but do not penetrate the respiratory mucosa (Peeters et al., 2005).

We hypothesised that in feline URT aspergillosis, given its more invasive nature,
infection would be accompanied by release of GM into the circulation. The aim of this study was to evaluate serum GM detection for the diagnosis of feline URT aspergillosis.

**Materials and Methods**

Clinical data and serum were collected prospectively from cats with diagnosed URT aspergillosis (Group 1 – URT Aspergillosis), cats with URT signs not attributable to aspergillosis (Group 2 - URT Other), cats treated with β-lactam antibiotics (Group 3 - β-lactam) and healthy cats (Group 4a- Young Healthy and Group 4b - Adult Healthy group). Samples were collected with informed owner consent, according to the guidelines of the Animal Ethics Committee of the University of Sydney (AEC Ref No. N00/3-2009/2/4961). Briefly, whole blood (1-2mL) was collected by jugular venipuncture into a plain tube, allowed to clot at room temperature, centrifuged and the serum decanted into a sterile microcentrifuge tube. Samples were stored at -80°C until analysis. Quantification of serum GM in all samples was performed by one of the authors (JW), who was blinded to the identity of the samples. The assay was performed according to the manufacturer’s instructions using a one-stage, immunoenzymatic sandwich ELISA (Platelia™ Aspergillus EIA, Bio-Rad, Marnes-la-Coquette, France) including pre-treatment EDTA-heat extraction to dissociate immune complexes. The assay utilizes a rat monoclonal antibody, EB-A2, which binds to the galactofuran epitope of the GM antigen, specifically to the (1→5)-β-D-galactofuranosyl (galf) side chains (Swanink et al., 1997).

Samples were run in duplicate in batches including a positive control, negative control, cut-off samples and a blank well. The two most common cut-off indices
reported in the human literature, optical density index (ODI) 0.5 and 1.5, were used to
determine positive results (Maertens et al., 2006). The mean reported intra-assay and
inter-assay coefficients of variation (CV) of the Platelia™ Aspergillus EIA are 6.6%
and 17.7% respectively for low positive samples (ODI < 0.8) and 4.9% and 17.7%
respectively for high positive samples (ODI > 2) (manufacturer’s information).

Cats

Group 1 – URT Aspergillosis

Inclusion criteria for cats with URT aspergillosis were the identification of fungal
hyphae on cytology or histology of tissue from the sinonasal cavity or orbit, a positive
fungal culture and molecular identification on the basis of PCR and sequencing of the
ITS, β-tubulin and calmodulin gene regions (Barrs et al., 2012b). Samples were
collected before antifungal therapy and in a subset of cats serial samples were also
collected after diagnosis during antifungal therapy. Where more than 1 sample was
collected, only 1 sample from each cat collected at diagnosis was included in the
evaluation of the ELISA. Cats were classified as having SNA or SOA based on the
presence (SOA) or absence (SNA) of a retrobulbar mass on computed tomography or
magnetic resonance imaging at diagnosis. Signalment, history, physical examination
findings, intercurrent disease and results of culture and molecular identification results
were recorded.

Group 2 – URT Other

Inclusion criteria for cats with URT disease not attributable to aspergillosis were (i)
consistent clinical signs e.g. sneezing, nasal discharge (ii) absence of fungal hyphae
on cytology or histology of tissue collected from the sinonasal cavity, and/or (iii)
serological, histopathological or endoscopic diagnosis of another URT disease. Standard diagnostic investigations included latex antigen cryptococcal serology (CALAS® Meridian Biosciences), upper airway endoscopy, advanced imaging (CT/MRI) and biopsy. Only cats with a definitive diagnosis were included in this group. Samples were collected prior to the initiation of therapy.

**Group 3 – β-lactam**
Inclusion criteria were (i) the absence of respiratory tract signs and (ii) ongoing treatment with amoxicillin-clavulanate or ticarcillin-clavulanate which had been initiated at least 24 hours earlier.

**Group 4 – Healthy Cats**
Healthy cats presented to the Valentine Charlton Cat Centre for routine desexing, vaccination or annual health check were included if there was no evidence of recent or current clinical disease or medication based on history and physical examination. This group was further stratified according to age, as follows; Group 4a, 1 year of age and under, and Group 4b, older than 1 year.

**Statistical Analyses**
Statistical analyses were performed using GraphPad Prism® versions 5.04 (GraphPad Software, Inc. 2010). Age and GM ODI were compared between groups 1 to 4 using Kruskal-Wallis and Dunn’s Multiple Comparison Tests. Mann-Whitney tests were used to compare the median ODIs of Group 1 with different forms of disease and different causative agents.
Three different negative control groups were used when evaluating the specificity of the ELISA for the diagnosis of feline URT aspergillosis. The first control group comprised all the aspergillosis-negative cats (Groups 2, 3, 4a and 4b). A second evaluation of specificity was performed omitting groups 3 and 4a, which were predicted to have frequent false positive results. The third control group compromised only Group 2, the cases most likely to be tested in a clinical situation. For each cut-off ODI and control group, the sensitivity and specificity were calculated with 95% exact confidence intervals using a contingency table.

**Results**

**Cats**

There were 13 cats in Group 1, 15 in Group 2, 14 in Group 3 and 44 in Group 4 including 28 in Group 4a and 16 in Group 4b. Serum samples were collected from Group 1 cats before treatment, including 6 neutered males and 7 neutered females ranging in age from 2 to 14 years (mean 7y, median 5y) (Table 1). Two to three serial serum samples were collected from 3 Group 1 cats (Table 2). All 15 Group 2 cats were neutered (8 females, 7 males) and ranged in age from 2 to 20 years (mean 9.7y, median 10y). URT diseases in Group 2 cats included neoplasia (6), lymphoplasmacytic rhinitis (4), cryptococcosis (2), laryngeal paralysis (2) and nasopharyngeal stenosis (1). All 14 cats in Group 3 were neutered (7 females, 7 males) and ranged in age from 1 to 16 years (mean 10.7y, median 12 y). Diagnoses in Group 3 cats included enteropathies (3), urinary tract infection (3), pancreatitis (2), neoplasia (2), hepatic lipidosis (1), tooth root abscess (1), pemphigus foliaceous (1) and cat bite abscess (1). All 28 cats in Group 4a were entire (17 females, 11 males) and 1 year of age or less (mean 0.5y, median 0.4y). The 16 cats in Group 4b included
12 females (2 entire, 10 neutered) and 4 neutered males ranging in age from 1.3 to 10 year (mean 4.8y, median 4y). There was no significant difference in age between Groups 1, 2, 3 and 4b. The median age of Group 4a was significantly different to all other groups (P<0.05).

**Galactomannan Quantification**

There was no significant difference between groups regarding the GM ODI (P=0.31) (Figure 1). The median GM ODI was 0.32 (range 0.14-5.34) in Group 1, 0.38 (0.15-6.13) in Group 2, 0.44 (0.17-6.10) in Group 3, 0.48 (range 0.15-6.66) in Group 4a and 0.52 (range 0.16-5.36) in Group 4b. Thirty-five cats had a GM ODI greater than 0.5 and 19 cats had an ODI greater than 1.5.

A serum GM ODI of greater than 0.5 was detected in 3 (23%) Group 1 cats, 4 (27%) Group 2 cats, 7 (50%) cats Group 3 cats, 13 (46%) Group 4a cats and 8 (50%) Group 4b cats. A serum GM ODI of greater than 1.5 was detected in 3 (23%) Group 1 cats, 1 (7%) Group 2 cat, 4 (29%) Group 3 cats, 9 (32%) Group 4 a cats and 2 (13%) Group 4b cats. The sensitivity and specificity of serum GM measurement in the diagnosis of feline URT aspergillosis using different negative control groups is described in Table 3.

Of the 13 cats with URT aspergillosis, 6 (46%) had SNA (median ODI = 0.34) and 7 (53.8%) had SOA (median ODI = 0.27) (Table 1). There was no significant difference in the median serum GM ODI between these groups (P=0.95). Three URT aspergillosis cats were infected with *Aspergillus fumigatus*, one with *Aspergillus lentulus*, one with *Aspergillus thermomutatus/Neosartorya pseudofischeri* and 8 cats
with a novel *Aspergillus* species (Barrs et al., 2012b). The only cats with GM ODI greater than twice the cut-off level were infected with the novel *Aspergillus* spp. (Table 1) although there was no statistically significant difference between the median ODI of all cats infected with the novel *Aspergillus* spp. (median ODI=0.29) and the other Group 1 cats (median ODI = 0.34, P=0.52). All cats tested for feline immunodeficiency virus and feline leukaemia virus (n=8) were seronegative.

Three cats (A, B and C) from Group 1 had serum samples available at diagnosis and after the initiation of treatment (Table 2). All 3 had SOA caused by a novel *Aspergillus* species. In Case A GM was negative both at diagnosis and during treatment. Case B had progression of clinical signs and extension into the CNS despite aggressive antifungal therapy. The second serum sample was collected shortly before euthanasia. Case C had a high GM ODI at presentation that decreased slightly after commencing antifungal therapy but increased again shortly before the cat was euthanased due to disease progression.

**Discussion**

The diagnostic sensitivity and specificity of a laboratory test describes its ability to identify affected and non-affected subjects respectively. In practice, discrimination by a single test is rarely absolute and a cut-off value is selected for a continuous variable, such as ODI, to maximise performance. Receiver operating characteristics (ROC) analysis can be used to evaluate diagnostic performance and this is the preferred method to determine the optimum cut-off value (Kaufmann et al., 2005). In this study, the median ODI for the URT aspergillosis group was not significantly different to the control groups, meaning that ROC analysis would not be meaningful. For this
reason, contingency analysis was used for evaluation of serum GM for the diagnosis of feline URT aspergillosis, using published ODI cut-off values to determine sensitivity and specificity (Maertens et al., 2006).

Specificity of the assay in this study was highest at an ODI of 1.5. The high frequency of false positive results in certain groups impacted the calculation of specificity. When all unaffected cases (Groups 2, 3 and 4) were considered together, the specificity using a cut-off ODI of 1.5 was 78% (Table 3). However, by removing Group 3 and 4a cats from the analysis, the specificity of the ELISA was markedly improved to 90% (cut-off index 1.5). Among the 15 Group 2 cats, the most clinically relevant control group, a single false positive result was returned from a cat with nasal cryptococcosis. Cryptococcosis is associated with high false positive GM results in human and canine patients (Xavier et al., 2009, Garcia et al., 2012). It is thought that *C. neoformans* galactoxylomannan contains cross-reactive epitopes that mimic *Aspergillus* GM (Dalle et al., 2005). Clinically, the exclusion of cats with a positive galactomannan titre that are infected with *C. neoformans* can be identified using the commercially available non-invasive latex cryptococcal antigen test.

The high frequency of false positive result among Group 3 and 4a cats in this study parallels findings in human studies. False-positives were recorded in 44% of paediatric patients, 83% of premature neonates and in 47% of human patients on amoxicillin-clavulanate therapy (Herbrecht et al., 2002, Siemann et al., 1998, Zandijk et al., 2008). It has been proposed that since the *Penicillium* spp., from which β-lactam antibiotics are manufactured produce GM, its introduction into the antibiotic
during preparation is the likely cause of this erroneous result (Hope et al., 2005).

These results differ from those seen in dogs in which 3/18 dogs treated with penicillins but without aspergillosis had positive galactomannan (Garcia et al., 2012). The sensitivity of the Platelia™ *Aspergillus* EIA for diagnosis of feline aspergillosis was poor at 23%. Reducing the ODI cut-off from 1.5 to 0.5, which would favour increased sensitivity over specificity, and which may have been acceptable for clinical application as a screening test to identify cases appropriate for further diagnostics, had no effect on the sensitivity. Several factors may have contributed to the failure to identify all Group 1 cats. In humans, the sensitivity of GM ELISA is typically greater than 90% in neutropenic patients (Hachem et al., 2009,) while in patients with solid-organ transplants or underlying pulmonary diseases, who are generally not neutropenic, the sensitivity is less than 30% using an ODI cut-off of 0.5 (Kitasato et al., 2009, Pfeiffer et al., 2006). The inability to detect circulating GM in immunocompetent patients is, in part due to antigen clearance or complexing by circulating anti-*Aspergillus* antibodies (Herbrecht et al., 2002). The sensitivity of GM detection is lower in patients with circulating antibodies at the onset of *Aspergillus* infection compared with seronegative patients (Herbrecht et al., 2002). Similar to the majority of reports of feline URT aspergillosis, none of the Group 1 cats in this study was known to have immunosuppressive disease (Barrs et al., 2012a). Sample EDTA-heat extraction pre-treatment to dissociate immune complexes could cause degradation of antigen which would reduce sensitivity (Menninck-Kersten et al., 2004). Alternative methods of complex dissociation such as addition of dithiothreitol or a protease (pronase) (Stockman & Roberts 1983) were not investigated.
While we hypothesised that the invasive nature of SOA would result in GM release into the circulation, the degree of angioinvasion in these immunocompetent cats may be less than that in neutropenic humans with invasive aspergillosis. Further the granulomatous inflammatory response that occurs in SOA (Barachetti et al., 2009, Barrs et al., 2012a, Giordano et al., 2010, Smith & Hoffmann 2010) could prevent GM release. Finally, a local environment deficient in oxygen and glucose can restrict fungal growth and GM release (Mennink-Kersten et al., 2004). This mechanism could be operating in areas of infarction and necrosis seen in the orbital and paranasal granulomas from cats with SOA (Barrs et al., 2012a) and may contribute to reduced circulating GM in the later stages of SOA.

In vitro studies have shown that the amount of GM released by *Aspergillus* species is species-dependent (Swanink et al., 1997). All of the Group 1 cats that were positive on the serum GM ELISA were infected with the same novel *Aspergillus fumigatus*-mimetic species which is closely related to *Aspergillus viridinutans* (Barrs et al, 2012b). One of these cats had SNA at diagnosis. Invasive sinonasal mucosal infection could account for the positive GM result in this case. In one study the sensitivity of the GM assay was significantly higher for IA due to non-*fumigatus Aspergillus* species than for patients with IA due to *A. fumigatus* infection (Hachem et al., 2009). Pretreatment concentration of serum GM based on retention above a 50-kDa cut-off filter has been found to markedly increase the sensitivity of the Platelia™ *Aspergillus* EIA in human sera (Mennink-Kersten et al., 2008). This modification is worthy of further investigation for feline samples.
Limited samples were available for longitudinal study of serum GM in individual patients. In case A, serum GM values were consistently negative on ELISA, even when the cat came out of remission. It should be noted that this cat had the best response of any of the SOA cases treated and is in remission at the time of writing, 5 years from first diagnosis. It may be that the negative serum GM in case A is a reflection of a superior immune response compared with other cases. GM levels in case B decreased dramatically during aggressive antifungal therapy. Concurrent antifungal treatment is a recognised cause of false-negative results in humans with invasive aspergillosis (Hope et al., 2005). Unfortunately, the decrease in GM did not correlate with clinical improvement and case B was euthanased because of progressive disease with central nervous system involvement. Case C had a persistently high GM and was euthanased due to disease progression despite aggressive treatment. This result may reflect an inadequate immune response in this patient.

The study of naturally acquired disease in a clinical setting is arguably the most relevant source of data where the goal is to diagnose and treat field cases. The stringent criteria used to define the affected population here provide a robust gold standard for diagnostic evaluation of the assay. Although samples numbers were limited, the group of affected cats studied represents 28% of the total reported cases of feline URT aspergillosis. The reported intra-assay CV for the Platelia™ *Aspergillus* EIA is relatively low indicating that this is unlikely to be a significant limitation of the assay. The inter-assay CV is slightly above the recognised acceptable limit for a biological assay (10-15%). However, despite this the assay is used extensively in
human medicine and has been recently shown to be of diagnostic benefit in the dogs with systemic aspergillosis (Garcia et al., 2012).

In conclusion, GM quantification by ELISA is not a reliable test for the early, non-invasive diagnosis of feline URT aspergillosis. However, in cats with upper respiratory signs it has good specificity. Investigation of serological detection of anti-Aspergillus antibodies for early non-invasive diagnosis of feline URTA is warranted.

Acknowledgements

The authors thank Dr Catriona Halliday and Sue Sleiman from Centre for Infectious Diseases and Microbiology Westmead Hospital, Westmead, Australia for advice on the ELISA used in this study.

This study was funded by the Feline Health Research Fund (FHRF). The FHRF was not involved in the study design, analysis or data interpretation.

References


### Table 1
Fungal species and ODI for URT Aspergillosis cats.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Breed</th>
<th>Form</th>
<th>FIV/FeLV</th>
<th>Fungal Species</th>
<th>ODI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>FN</td>
<td>Persian</td>
<td>SNA</td>
<td>Not tested</td>
<td><em>A. fumigatus</em></td>
<td>0.426</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>MN</td>
<td>DLH</td>
<td>SNA</td>
<td>Negative</td>
<td><em>A. fumigatus</em></td>
<td>0.337</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>FN</td>
<td>Persian</td>
<td>SNA</td>
<td>Not tested</td>
<td><em>A. lentulus</em></td>
<td>0.335</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cross</td>
<td>SNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>MN</td>
<td>Persian</td>
<td>SNA</td>
<td>Negative</td>
<td><em>A. fumigatus</em></td>
<td>0.141</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>MN</td>
<td>Rag Doll</td>
<td>SNA</td>
<td>Not tested</td>
<td><em>A. thermomutatus/ Neosartorya pseudofischeri</em></td>
<td>0.140</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>MN</td>
<td>DSH</td>
<td>SNA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>5.339</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>MN</td>
<td>Himalayan</td>
<td>SOA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>4.961</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>FN</td>
<td>DSH</td>
<td>SOA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>0.318</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>FN</td>
<td>DSH</td>
<td>SOA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>0.248</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>MN</td>
<td>DSH</td>
<td>SOA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>5.318</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>FN</td>
<td>Persian</td>
<td>SOA</td>
<td>Not tested</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>0.265</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>FN</td>
<td>DSH</td>
<td>SOA</td>
<td>Not tested</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>0.206</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>FN</td>
<td>Cornish Rex</td>
<td>SOA</td>
<td>Negative</td>
<td><em>Aspergillus</em> spp. nov.</td>
<td>0.155</td>
</tr>
</tbody>
</table>

DSH, Domestic shorthair; DLH, Domestic longhair; SNA, sino-orbital aspergillosis; SNA, sino-nasal aspergilosis; ODI, optical density index
Table 2

Galactomannan ODI for URT Aspergillosis cats with multiple samples.

<table>
<thead>
<tr>
<th>Signalment</th>
<th>Collection Date</th>
<th>Clinical Data</th>
<th>ODI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case A</td>
<td>12.4.2007</td>
<td>No clinical signs, off treatment</td>
<td>0.306</td>
</tr>
<tr>
<td>2y FN Cornish Rex</td>
<td>1.12.2007</td>
<td>No clinical signs, off treatment</td>
<td>0.386</td>
</tr>
<tr>
<td></td>
<td>14.10.2009</td>
<td>Clinical Signs (relapse), no</td>
<td>0.155*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.2.2010</td>
<td>No clinical signs, on treatment</td>
<td>0.281</td>
</tr>
<tr>
<td>Case B</td>
<td>7.3.2008</td>
<td>Clinical Signs, no treatment</td>
<td>5.318*</td>
</tr>
<tr>
<td>3y MN DSH</td>
<td>28.4.2008</td>
<td>On treatment, clinical signs</td>
<td>0.367</td>
</tr>
<tr>
<td>Case C</td>
<td>26.2.2007</td>
<td>Clinical Signs, no treatment</td>
<td>4.08*</td>
</tr>
<tr>
<td>2y MN Himalayan</td>
<td>2.3.2007</td>
<td>Clinical Signs, on treatment</td>
<td>3.728</td>
</tr>
<tr>
<td></td>
<td>10.4.2007</td>
<td>Clinical Signs, on treatment</td>
<td>4.961</td>
</tr>
</tbody>
</table>

FN, female neutered; MN, male neutered; DSH, domestic shorthair; ODI, optical density index; * ODI used in statistical calculations
Table 3

Sensitivity and specificity of serum galactomannan measurement for diagnosing URT aspergillosis in cats evaluated by comparing confirmed URT aspergillosis cats (Group 1; n = 26) with different negative control groups*.

<table>
<thead>
<tr>
<th>Negative Control Groups</th>
<th>0.5 ODI cut-off</th>
<th>1.5 ODI cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>2, 3 &amp; 4</td>
<td>23.08% (5.04-53.81)</td>
<td>56.16% (44.05-67.76)</td>
</tr>
<tr>
<td>2 &amp; 4b</td>
<td>23.08% (5.04-53.81)</td>
<td>61.29% (42.19-78.15)</td>
</tr>
<tr>
<td>2</td>
<td>23.08% (5.04-53.81)</td>
<td>73.33% (44.90-92.21)</td>
</tr>
</tbody>
</table>

CI, confidence interval; ODI, optical density index

* Group 2 (URT other) – other URT diseases (n=15); Group 3 (β-lactam) – cats treated with β-lactam antibiotics for non-respiratory tract disease (n=14); Group 4a – healthy young cats (≤1 y of age, n=28); Group 4b – healthy adult cats (>1 y of age, n=16).
Figure 1 Serum Galactomannan Measurement

GALACTOMANNAN OPTICAL DENSITY INDEX

URT Aspergillosis  URT Other  Beta-Lactam  Young Healthy  Adult Healthy

GROUPS

1.5 OD Cut-Off
0.5 OD Cut-Off