EVALUATION OF THE FUJIFILM™ WAKO β-D-GLUCAN ASSAY IN PATIENT SERUM FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS AND INVASIVE CANDIDIASIS

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INTRODUCTION

Invasive aspergillosis (IA) and invasive candidiasis (IC) are fungal diseases that can carry a high morbidity and mortality, especially in those patients who are immunosuppressed (1). Early diagnosis of fungal disease is key to implementing early treatment and improving patient prognosis. Currently in MMUH, serum β-D-Glucan (BDG) is available as an external referral test only (average TAT 109.5 days). Expanding our current repertoire of tests to include BDG, would greatly aid in the early diagnosis of IA or IC. Delay or misdiagnosis of IA or IC can result in drug toxicity due to inappropriate treatment, high medical expenses and mortality. Given the complexity of invasive fungal diseases, a number of tests are required for correct diagnosis. Therefore implementation of another test that can quickly and accurately aid in the diagnosis of fungal diseases would have great benefits for overall patient care, whilst limiting unnecessary use of antifungal therapy within the hospital.

In MMUH at present, IA and IC are identified by a combination of clinical suspicion, microbiological culture, calcofluor/KOH results, histological specimens, and radiological imaging. Bronchoalveolar lavage galactomannan, serum galactomannan or serum BDG are not readily accessible and available as external referral tests only. The Fujifilm Wako™ BDG is based on limulus amebocyte lysate cascade reactions in patient serum, and is measured by a kinetic turbidimetric method.

PURPOSE OF STUDY

•To evaluate the Wako™ β-D-Glucan assay in the laboratory and its' implications for clinical practice.
• To assess the contribution this test can make to patient diagnosis

MATERIALS AND METHODS

Figure 1: Study design

Step 1: Pre-treatment:

100ul of plasma or serum are added to 900ul of pretreatment reagent. After mixing, the sample is heated at 70°C for 10 minutes and cooled at -20C for 10minutes. Both positive and negative controls are included in a run once weekly. They are treated in the same manner.

Step 2: Measurement:

200ul of the pretreated sample is transferred into the LAL reagent vial. After mixing, the sample is inserted into the Toxinometer. The measurement starts immediately and takes 90 minutes.

INTERPRETATION OF RESULTS

Manufacturers guidelines state that a cut off value of 11pg/mL and above indicates a positive BDG result. With clinical consideration, it was decided to lower our cut off value to 7pg/mL. Therefore, any result with a pg/mL value of seven or more was considered to be a positive BDG result.

All samples were also sent to our reference laboratory for duplicate testing and comparative analysis. The results were analyzed in conjunction with results from previous microbiological cultures, serum and/or bronchoalveolar lavage galactomannan, OLM™ lateral flow device, calcofluor/KOH, histological specimens, radiological imaging and clinical details where available. EORTC criteria (1) were used to categorize patients into proven, probable or possible IA. A diagnosis of invasive candidiasis was either proven, possible or out-ruled.

RESULTS AND CONCLUSIONS

Of **41 tests**, **16** were for the diagnosis of **IA**, **13** for **IC** , and **12** were in patients without a suspicion or diagnosis for a fungal infection; **true negatives**.

Table 1: Summary of results

	Invasive aspergillosis	Invasive candidiasis
Sensitivity	100%	80%
Specificity	81.9%	100%
PPV	71.4%	100%
NPV	100%	88.9%

DISCUSSION

INVASIVE ASPERGILLOSIS:

•16 patients had IA as part of their differential diagnosis. Using EORTC criteria, of the 16 patients, 0 were proven, 5 considered probable, 6 had the diagnosis of IA out ruled and 5 were unfulfilled.

•Of the 5 cases considered probable, the Wako™ β-D-Glucan was positive in all.

•In the 6 cases in whom a diagnosis of IA was out ruled, 6 had a negative Wako™ β-D-Glucan.

•In five cases there was a non-specific radiological abnormality and a BDG was sent as a screening tool. In these cases, 3 were BDG negative thought to be true negatives and 2 were positive, thought to be false positive,

•In total, of 16 tests, 5 were true positives, 9 were true negatives, 2 were falsely positive and 0 false negatives.

INVASIVE CANDIDIASIS:

- 11 patients had a Wako™ BDG for investigation for IC. In 3 patients with proven IC, all were BDG positive. One patient was categorized as possible IC, where one test was positive and one negative, thought to be a false negative result. Six patients had risk factors for IC however all were subsequently not proven and all 6 patients had a negative test, one of whom had two negative tests. Finally one patient had a previous diagnosis of IC that had been treated and had a BDG for surveillance, which was negative.
- Of 13 tests in 11 patients, there were 4 true positives, 8 true negatives, 1 false negative and no false positives.

TRUE NEGATIVES:

- Finally 12 tests were undertaken in a patient cohort in whom no diagnosis of a fungal infection was made. Twelve of 12 of these tests were negative.

CONCLUSION

The Wako™ β-D-Glucan is considered a valuable diagnostic tool in MMUH due to its' robust nature and user friendliness, allowing both single and sequential testing of samples. Based on results of this study, the Wako™ β-D-Glucan assay will be introduced in MMUH, for patients with suspected IA or IC. In doing so, the turn around time for our BDG results will be significantly reduced.

REFERENCES

1. De Pauw B et al (2008) Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group, *Clinical Infectious Diseases*, Volume 46, Issue 12, 15 June 2008, Pages 1813–1821

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