CORRESPONDENCE

The impact of indirect immunofluorescence screening dilution on the positivity of gastric parietal cell antibodies

Sir,

Pernicious anaemia (PA) is an autoimmune disease characterised by a decrease in the amount or function of intrinsic factor, resulting in the malabsorption of vitamin B12.¹ Intrinsic factor, which is produced by the parietal cells of the stomach lining, avidly binds dietary vitamin B12 and is essential for its absorption. PA is characterised by histologically proven atrophic body gastritis, peripheral blood examination showing megaloblastic anaemia with hypersegmented neutrophils, cobalamin deficiency and antibodies to gastric parietal cells and/or intrinsic factor.¹

PA affects both sexes and is usually seen in adults over 30 years of age.¹ In PA patients, 55–90% have identifiable gastric parietal cell antibodies (GPCA),^{2,3} the prevalence of which appears to be influenced by ethnicity and age.³ These autoantibodies against the hydrogen transporting enzyme H⁺/K⁺ ATPase of gastric parietal cells induce the destruction of the cells and thus result in absent or reduced intrinsic factor production.^{4,5} In addition, 40–80% of PA patients also develop antibodies against intrinsic factor. Intrinsic factor antibodies (IFA) bind to the vitamin B12-binding site of intrinsic factor and subsequently inactivate the vitamin B12 absorption function of intrinsic factor. IFA can be found in isolation or in addition to GPCA.²

An increased prevalence of PA has been linked to a number of other autoimmune conditions such as Addison's disease, Graves' disease, Myasthenia gravis, Type 1 diabetes, Sjögren's syndrome, Hashimoto's disease and coeliac disease.^{1,6}

The identification of GPCA has historically been performed using indirect immunofluorescence (IIF), with diluted patient sera incubated on rodent gastric tract tissue and detected with fluorescein isothiocyanate (FITC)-labelled anti-human IgG antiserum. Due to the highly variable manual methodologies and subjective nature of interpretation, the sensitivity and specificity of GPCA varies. While many authors note a reasonable sensitivity in PA patients (85–90%), low specificity has been described and antibody positivity reported in a variety of other diseases including atrophic gastritis without megaloblastic anaemia as well as in various autoimmune disorders.⁶

Finding the optimal screening dilution to maximise sensitivity and specificity is essential, however in practice it is highly problematic. In 2018, two samples provided by The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) Tissue Antibody Program (TA17.06 and TA17.07) highlighted the variation in positivity (sensitivity) in the reporting of GPCA.

In the first sample (TA17.06), serum was collected from a 50-year-old female with PA and Hashimoto's thyroiditis. Sixty-two laboratories reported results for this sample. Of these, 43 (69%) reported a positive result for GPCA, 16 (26%) reported negative and 3 (5%) reported equivocal positivity. The majority of participants, 54/62 (87%) reported

using an IIF method, while 8 (13%) were using an automated method [enzyme-linked immunosorbent assay (ELISA), EliA (ThermoFisher, USA) or FIDIS (Theradig, France)].

A similar discrepancy was seen in the second sample (TA17.07) from a 75-year-old female with PA and reactive gastritis, confirmed by gastric antrum biopsy. In this instance 64 laboratories reported results for this sample. Of these, 42 (66%) reported a positive result for GPCA, 17 (27%) reported negative and 5 (8%) reported an equivocal result. Both samples were confirmed as positive against the target antigen identified by GPCA (the hydrogen transporting enzyme H⁺/K⁺ ATPase of gastric parietal cells at a dilution of 1:101 by ELISA; Aesku.Diagnostics, Germany).

To further understand the variation of results reported by laboratories, a survey was sent to RCPAQAP participants asking them details of their methodology for GPCA including screening dilution. Results of the survey were then linked to individual performance over the two samples.

Forty-four laboratories responded to the survey, with 41 (93%) using IIF and 3 (7%) using an automated method (EliA performed on the Phadia; ThermoFisher). Screening dilutions were reported by 39 IIF users. Survey responses were then compared to the results submitted by each laboratory for samples TA17.06 and TA17.07 and an overall positivity rate compared to screening dilution calculated (Table 1).

Analysis showed that laboratories using a screening dilution of 1:10 for GPCA had a higher positivity rate (sensitivity) than laboratories screening at higher dilutions. Manufacturers of tissue slides were also contacted to ascertain their recommendations for screening dilutions. Of the three manufacturers that responded one (Euroimmun) recommend a screening dilution of 1:10, while two (ImmunoConcepts and Inova) recommended 1:20.

These results show that laboratories who are using higher screening dilutions may be doing so at the expense of analytical sensitivity, which may have an impact on patient diagnosis. It should be noted that other factors such as substrate choice (rat versus mouse), conjugate source and dilution, incubation times, the condition, age and illumination of the microscope used and the experience of the reader also have the potential to impact on the sensitivity and specificity of results.

Table 1 Screening dilutions reported for laboratories using an indirect immunofluorescence (IIF) method

Screening dilution	No. laboratories	Combined positivity rate of RCPAQAP samples TA17.06 and TA17.07 ^a
1:10	9	94%
1:20	17	75%
1:40	10	78%
1:80	2	75%
Other	1	N/A

^a Results from The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) Tissue Antibody Program samples TA17.06 and TA17.07 were compared to screening dilution and reported as a positivity rate.

Considering these findings, we recommend that laboratories review their current methodologies to ensure that the IIF screening dilution used for the detection of GPCA has optimal sensitivity and specificity for their methodology and microscope.

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Candida duobushaemulonii sepsis and Candida auris co-isolation following hospitalisation in Vietnam

Sir,

Candida auris is an emerging pathogen linked with health-care associated outbreaks.¹ *Candida duobushaemulonii* is closely related to *C. auris* and belongs to the *C. haemulonii* complex. It often exhibits elevated minimum inhibitory concentrations (MICs) to azoles and amphotericin B.^{2,3} An unexpected number of invasive *C. duobushaemulonii* isolates reported in Panama in 2016–2017 raised concern regarding emergence of *C. duobushaemulonii* as a cause of highly transmissible multidrug resistant infection.² We report a patient with two antifungal resistant *Candida* species: *C. duobushaemulonii* bloodstream infection and *C. auris* colonisation after transfer from a hospital in southern Vietnam.

In June 2019, an 85-year-old man was transferred to Melbourne, Australia from Vietnam. He had been

hospitalised 2 months prior while visiting Vietnam with altered conscious state and hyponatraemia in the setting of an *Escherichia coli* urinary tract infection. His medical history included non-insulin dependent diabetes and IgA nephropathy with a baseline creatinine of 200 µmol/L. His stay in Vietnam required prolonged intensive care unit admission complicated by cardiac arrest after acute coronary syndrome, anuric renal failure, gastrointestinal haemorrhage, and carbapenem resistant *Klebsiella pneumoniae* pneumonia and bloodstream infection. His antimicrobial use included colistin, meropenem, vancomycin and caspofungin.

On arrival to Melbourne, there was no evidence of ongoing infection and his antimicrobials were ceased. All central venous access devices were replaced. A groin and axilla swab, catheter urine and endotracheal aspirate were taken for *C. auris* screening. He was placed in contact isolation.

The *C. auris* screening specimens were cultured on Brilliance Candida Agar (Oxoid, UK). After 72 h incubation pink colonies were isolated and identified as *C. auris* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) with a score of >2.0 (MALDI Biotyper version 3.1, database MBT 7854 MSP library RUO; Bruker Daltonik, Germany). Identity was confirmed by sequencing of the internal transcribed spacer region (ITS2) with primers in the conserved flanking 5.8S and 28S region, with 99.2% match at 100% coverage to *C. auris* with NCBI accession number MF817727.1 (type strain DSM21092). *Candida duobushaemulonii* was not isolated. Antifungal susceptibility testing with Sensititre YeastOne plate (TREK Diagnostic Systems, UK) demonstrated high MICs to fluconazole (MIC 128 µg/mL) and amphotericin (MIC 2 µg/mL), and a low MIC to micafungin (0.12 µg/mL). These may be tentatively interpreted as resistance to fluconazole and amphotericin and susceptibility to micafungin based on CDC interpretive criteria.⁴

On day 9 after transfer, he developed a fever with increasing vasopressor requirements. After discussion with his family, treatment was withdrawn due to poor neurological recovery.

A yeast was isolated on day 3 of incubation from a blood culture taken from the central venous catheter (BACTEC 9240 system; Becton Dickinson, USA). It was identified as *C. duobushaemulonii* by MALDI-TOF MS (Bruker Daltonik) with a confidence score of >2.0. The identity was confirmed by sequencing of the ITS2 region which matched 100% at 100% coverage with NCBI accession number NR_130694.1 (type strain CBS7789). It was subsequently cultured on Brilliance Candida Agar and yielded beige colonies. Susceptibility testing with Sensititre YeastOne demonstrated a fluconazole MIC of 64 µg/mL, micafungin MIC 0.06 µg/mL and amphotericin B MIC 0.5 µg/mL. The isolate was sent to another clinical laboratory and tested on Vitek MS MALDI (bioMérieux, France) and Vitek2 Yeast ID card which yielded identification of *C. duobushaemulonii* with a confidence value of 99.9% and 97% probability, respectively. Colony colour on CHROMagar Candida medium (Becton Dickinson) was cream with a pale pink hue in the primary inoculum (Fig. 1).

Candida duobushaemulonii has previously been reported to be an uncommon cause of invasive infection in India, East