A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits

R C W Wong, R J Wilson, R H Steele, G Radford-Smith, S Adelstein

ORIGINAL ARTICLE

Aims: Tissue transglutaminase (tTG) is a major autoantigen recognised by IgA anti-endomysial antibodies (IgA EMA). Enzyme linked immunosorbent assays (ELISA) for IgA anti-tissue transglutaminase antibodies (IgA tTG) have therefore been developed as an alternative serological screening test to IgA EMA for coeliac disease (CD). The use of human tTG (h-tTG), as opposed to guinea pig liver tTG (gpl-tTG), in these assays has been reported to produce superior results. This study compared 13 commercial IgA tTG ELISA kits to ascertain their performance characteristics in the diagnosis of CD in patients with biopsy confirmed disease compared with controls. All patients and controls were adults aged 21 years or older.

Methods: Sera from the following groups of patients were tested in each kit: (1) 49 patients with CD confirmed on small bowel biopsies (all IgA EMA positive); (2) 34 patients with small bowel biopsies that were not consistent with CD; and (3) 30 patients with biopsy confirmed inflammatory bowel disease. All controls were negative for IgA EMA and were not IgA deficient. Sensitivities and specificities were determined using both the manufacturers’ recommended cut off points and receiver operating characteristic (ROC) analysis derived decision thresholds. The area under the curve (AUC) for each ROC plot was also calculated and compared between kits.

Results: In general, the h-tTG based IgA tTG ELISA kits demonstrated superior performance (especially specificity) compared with the gpl-tTG based kits, although 100% sensitivity and specificity (comparable to the IgA EMA assay) was obtained in only one recombinant h-tTG based kit.

Conclusions: The use of h-tTG in IgA tTG ELISA kits is generally, but not universally, associated with superior performance. Factors other than antigen source are important in determining kit performance.

Although many studies have concluded that the IgA tTG assay has comparable performance to the IgA EMA IIF assay, several have described false negative IgA tTG results in subjects with IgA EMA positive untreated CD.4–10,14,15,21,22,26,28–30,32,33,36,38 and false positive IgA tTG results in the absence of IgA EMA and CD.5–38

However, most of these studies used gpl-tTG, which has only about 81% homology with h-tTG.39 In contrast, the use of h-tTG has been reported to be associated with fewer false negative and false positive results, and an overall performance closely comparable or equal to the “gold standard” IgA EMA IIF assay.1,11–15,21,24,29,35,18,38,40

However, because none of these studies has compared gpl-tTG based ELISAs with two or more h-tTG-based ELISAs, it is unclear whether the use of h-tTG alone results in superior performance to the gpl-tTG-based assays.

We compared 13 commercial IgA tTG ELISA kits, seven gpl-tTG based and six h-tTG based (four recombinant h-tTG), in 49 IgA EMA positive adult patients with CD and 64 adult disease controls to establish the sensitivity and specificity of each kit.

Abbreviations: APTS, 2,2’-azino-bis-3-ethylbenzthiazolin-6-sulphonic acid; AU, arbitrary units; AUC, area under curve; BSA, bovine serum albumin; CD, coeliac disease; ELISA, enzyme linked immunosorbent assay; gpl-tTG, guinea pig liver tissue transglutaminase; HRP, horseradish peroxidase; h-tTG, human tissue transglutaminase; IBD, inflammatory bowel disease; IgA EMA, IgA anti-endomysial antibody; IgA tTG, IgA anti-tissue transglutaminase antibody; IIF, indirect immunofluorescence; PNP, para-nitrophenyl phosphate; ROC, receiver operating characteristic; TMB, 3, 3’, 5’, 5’-tetrathylbenzidine; tTG, tissue transglutaminase
Comparison of anti-tTG ELISA kits

Table 1

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Source of tTG</th>
<th>Calcium activated</th>
<th>Substrate</th>
<th>Incubation times (serum, conjugate) in minutes</th>
<th>Conjugate</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AESKULISA/Aesku.Lab Diagnostica (Wendelsheim, Germany)</td>
<td>Recombinant human, NS</td>
<td>1/100</td>
<td>30, 15</td>
<td>HRP antihuman IgA</td>
<td>TMB</td>
<td></td>
</tr>
<tr>
<td>The Binding Site (Birmingham, UK)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 30</td>
<td>Rabbit HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td></td>
<td>Recombinant human, NS</td>
<td>1/100</td>
<td>30, 30</td>
<td>Rabbit HRP antihuman IgA</td>
<td>TMB</td>
<td></td>
</tr>
<tr>
<td>Eurospital S.p.A (Trieste, Italy)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/25</td>
<td>60, 60</td>
<td>Sheep HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td>Genesis Diagnostics (Littleport, UK)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 30</td>
<td>Rabbit HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td>ImmuLisa/Immco Diagnostics Inc (Buffalo, New York, USA)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/50</td>
<td>60, 30</td>
<td>Alkaline phosphatase</td>
<td>PNPP</td>
</tr>
<tr>
<td>Immunopharmacology Research Diagnostics (Catania, Italy)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 30</td>
<td>HRP antihuman IgA†</td>
<td>TMB</td>
</tr>
<tr>
<td>QUANTA Lite/Inova (Diagnostics Inc, San Diego, California, USA)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 30</td>
<td>Goat HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td>Medizyme/Medipan Diagnostica GmbH (Selchow, Germany)</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/50</td>
<td>60, 30</td>
<td>Sheep HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td>Orgentec Diagnostika GmbH (Mainz, Germany)</td>
<td>Purified human NS</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 15</td>
<td>Rabbit HRP antihuman IgA</td>
<td>TMB</td>
</tr>
<tr>
<td>Varelisa/Pharmacia &amp; Upjohn Diagnostics GmbH &amp; Co (Freiburg, Germany)</td>
<td>Recombinant human, NS</td>
<td>Yes</td>
<td>1/100</td>
<td>30, 30</td>
<td>HRP antihuman IgA</td>
<td>TMB</td>
</tr>
</tbody>
</table>

†In the manufacturer’s kit insert, alkaline phosphatase was mentioned under “Principle”, but HRP was mentioned under “Reagent supplied.”

HRP, horseradish peroxidase; NS, not stated; PNPP, paranitrophenyl phosphate; TMB, 3,3′,5,5′-tetramethylbenzidine; tTG, tissue transglutaminase.

METHODS

Patients

One hundred and thirteen sera were selected from samples submitted to: Division of Immunology, Queensland Health Pathology Services, Royal Brisbane and Princess Alexandra Hospitals; Central Sydney Immunology Laboratory; and Department of Immunology, Sullivan Nicolaides Pathology. These comprised sera from the following patients who were aged 21 years or older: (1) 49 patients with typical histological changes of CD on small bowel biopsy,31 who had previously been found to have a positive IgA EMA, 38 of whom had never been on a gluten free diet, and 11 of whom were poorly compliant or non-compliant with the diet and had an abnormal small bowel biopsy close to the time of blood sampling; (2) 34 subjects who had been investigated with upper gastrointestinal fibroptic endoscopy and small bowel biopsy for possible CD and were found not to have histological changes consistent with CD (non-CD controls), with the following results on small bowel biopsy (no evidence of villous atrophy in all cases): normal duodenum (n = 27), duodenal ulcer (n = 3), dilated Brunner’s glands (n = 1), non-specific duodenitis (n = 1), fibrotic and thickened small bowel (n = 1), and gastric atrophy (n = 1); and (3) 30 subjects with biopsy confirmed inflammatory bowel disease (IBD controls).

All sera were retested for IgA EMA at the start of the study to ensure that the sera from patients with CD had not degraded during storage at −70°C. Total serum IgA values were also measured in all 64 non-CD and IBD control sera by nephelometry (Behring Diagnostics, Frankfurt, Germany). All 64 controls had values within the normal range for adults (1.24–4.16 g/litre), thus excluding IgA deficiency as a potential cause for negative results.

IgA EMA IIF assay

The IgA EMA assay was performed by IIF using cryostat sections of monkey oesophagus (The Binding Site, Birmingham, UK), as described previously32 at a screening dilution of 1/4. All slides were viewed by two independent observers and a positive or negative result was determined by consensus.

IgA tTG ELISA

The manufacturer’s instructions (table 1) were followed for all 13 IgA tTG ELISA kits. All specimens were tested in duplicate.

Bovine serum albumin and gelatin coated ELISA plates

To investigate the possibility of IgA anti-bovine serum albumin (BSA) antibodies producing false positive IgA tTG results, ELISA plates (Costar, Corning Inc, New York, USA) were coated with 250 pl of 5% BSA (Sigma Chemical Co, St Louis, Missouri, USA) or 1% gelatin (Bio-Rad, Hercules, California, USA). Serum diluted 1/100 in Tween/phosphate buffered saline was incubated for one hour at room temperature. After three washes, horseradish peroxidase (HRP) labelled goat antihuman IgA (Silenus Labs, Melbourne, Australia), at a dilution of 1/500, was added and the plates were incubated for one hour (room temperature). ABTS (2,2′-azino-bis-3-ethylbenzthiazolin-6-sulphonic acid) substrate (Medical Innovations, Sydney, Australia) was added for 15 minutes, and absorbances read at 405 nm.

Cut off values

Both the manufacturers’ recommended cut off values and decision thresholds determined by receiver operating characteristic (ROC) plots (see below) were used to calculate the sensitivity and specificity of each assay/kit. The IBD controls
were not used in the calculation of specificity because some had not undergone small bowel biopsy to exclude CD.

**ROC plot analysis**

ROC plot analysis was performed on each kit using the Accuroc software package (Accumetric Corporation, McGill University Health Centre, Montreal, Quebec, Canada) to determine a decision threshold and area under curve (AUC) estimation. The IBD controls were not included in the ROC analysis because some had not undergone small bowel biopsy to exclude CD. The AUC was calculated using the trapezoid rule. Comparisons between the AUCs of each kit were performed by the non-parametric method for correlated samples, as previously described by DeLong et al.

**RESULTS**

The IgA tTG values of the patients with CD and the non-CD and IBD controls measured with the 13 kits are shown in fig 1 (gpl-tTG based kits) and fig 2 (h-tTG based kits) with corresponding ROC curves and AUC estimations. The solid lines represent the manufacturers’ recommended cut off values and the broken lines represent the ROC plot analysis derived decision thresholds. The IBD controls were not included in the ROC analysis because some had not undergone small bowel biopsy to exclude CD. The AUC was calculated using the trapezoid rule. Comparisons between the AUCs of each kit were performed by the non-parametric method for correlated samples, as previously described by DeLong et al.

The recombinant h-tTG based Varelisa (Pharmacia & Upjohn Diagnostics, GmbH & Co, Freiburg, Germany) and UNEXA (Pharmacia & Upjohn Diagnostics, GmbH & Co, Freiburg, Germany) were not used in the calculation of specificity because some had not undergone small bowel biopsy to exclude CD.

![Figure 1](http://jcp.bmj.com/)

**Figure 1** IgA anti-tissue transglutaminase (tTG) antibody values of the patients with coeliac disease (CD), and the non-CD (SBX) and inflammatory bowel disease (IBD) controls in the seven purified guinea pig liver tTG based enzyme linked immunosorbent assay (ELISA) kits, with corresponding receiver operating characteristic (ROC) curves and area under curve (AUC) estimations. The solid lines represent the manufacturers’ recommended cut off values and the broken lines represent the ROC plot analysis derived decision thresholds. (A) The Binding Site, (B) Eurospital, (C) Genesis Diagnostics, (D) Immunopharmacology Research Diagnostics, (E) QUANTA Lite (Inova), (F) Medizyme (Medipan Diagnostica), (G) Immulisa (Immco).
purified erythrocyte h-tTG based QUANTA Lite (Inova Diagnostic Inc, San Diego, California, USA) kits performed best, with sensitivities of 100% and 98%, specificities of 100% and 100% (using the manufacturers’ cut off values), and AUC estimations of 1.000 and 1.000, respectively (fig 2; table 3).

Of the seven guinea pig liver tTG based kits (fig 1; table 2), the QUANTA Lite kit performed best, with 86% sensitivity and 100% specificity using the manufacturer’s cut off value of 20 arbitrary units/ml, and an AUC of 0.987. Applying the ROC analysis derived decision threshold of 14.1 arbitrary units/ml improved sensitivity to 92% but reduced specificity to 97%.

---

**Table 2** IgA EMA and IgA tTG results in patients with CD and controls using the manufacturers’ cut off points and ROC plot analysis derived decision thresholds for the seven guinea pig liver tTG based ELISA kits

<table>
<thead>
<tr>
<th>Assay type/Manufacturer</th>
<th>Manufacturer’s cut off point</th>
<th>ROC plot analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cut off</td>
<td>CD (sensitivity)</td>
</tr>
<tr>
<td>IgA EMA IIF/The Binding Site</td>
<td>1/4</td>
<td>49/49 (100%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/The Binding Site</td>
<td>4 U/ml</td>
<td>43/49 (88%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Eurospital</td>
<td>5 AU</td>
<td>48/49 (98%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Genesis Diagnostics</td>
<td>10 U/ml</td>
<td>47/49 (96%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/ImmuLisa</td>
<td>20 EU/ml</td>
<td>45/49 (92%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Immunopharmacology Research Diagnostics</td>
<td>25 AU</td>
<td>49/49 (100%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/QUANTA Lite</td>
<td>20 units/ml</td>
<td>42/49 (86%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Medizyme</td>
<td>25 U/ml</td>
<td>48/49 (98%)</td>
</tr>
</tbody>
</table>

Results equal to or greater than the cut off/threshold were considered positive. Specificity was calculated using only the non-CD controls (see text). AU, arbitrary units; CD, coeliac disease; ELISA, enzyme linked immunosorbent assay; IBD, inflammatory bowel disease; IgA EMA, IgA anti-endomysial antibody; IgA tTG, IgA anti-tissue transglutaminase antibody; IIF, indirect immunofluorescence; NA, not applicable; tTG, tissue transglutaminase; ROC, receiver operating characteristic.

---

*Figure 2* IgA anti-tissue transglutaminase (tTG) antibody values of the patients with coeliac disease (CD), and the non-CD (SBX) and inflammatory bowel disease (IBD) controls in the six human tTG based enzyme linked immunosorbent assay (ELISA) kits, with corresponding receiver operating characteristic (ROC) curves and area under curve (AUC) estimations. The solid lines represent the manufacturers’ recommended cut off values and the broken lines represent the ROC plot analysis derived decision thresholds. (A) AESKULISA (Aeskulab), (B) The Binding Site, (C) Eurospital, (D) QUANTA Lite (Inova), (E) Orgentec, (F) Varelisa (Pharmacia & Upjohn).
To exclude the possibility that some reactions to tTG were really reactions to blocking agents used in the ELISA kits, anti-BSA and antigelatin antibodies were determined (data not shown). Sera from one IBD control and two patients with anti-BSA and antigelatin antibodies were determined (data really reactions to blocking agents used in the ELISA kits, performance.

Other than antigen source are important in determining kit performance. h-tTG kits (figs 1, 2; tables 2, 3). This demonstrates that factors other than antigen source are important in determining kit performance.

In this comparison of 13 commercial IgA tTG ELISA kits, we found that the human tTG based kits tested generally demonstrated superior performance (especially specificity) to the gpl-tTG based kits (tables 2,3). However, three of the six h-tTG based kits (two recombinant h-tTG based) evaluated also produced false positive results in the absence of IgA EMA and CD, as previously reported.4,14,15 These findings raise the important issue of contaminants in gpl-tTG,24,25,38 which may contain other hepatic proteins.26,27 On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gpl-tTG extract (Sigma T5398; Sigma Chemical Co) used in several gpl-tTG based ELISAs5–7,11–13,15,18,20–25,32,33,38 contains multiple bands in addition to the 82–83 kDa tTG band,24,25,38 which only accounted for about 30% of the total protein.29,30 This may be partially overcome by further purification steps, and should be less of an issue with recombinant h-tTG.24,25,38

However, three of the six h-tTG based kits (two recombinant h-tTG based) evaluated also produced false positive results in the absence of IgA EMA and CD, as previously reported.4,14,15 Therefore, other explanations for false positive results are required.

The methods used to extract and purify tissue derived tissue transglutaminase (tTG), produce and process recombinant tTG, and then coat tTG on to ELISA wells may lead to alterations in the tertiary structure of tTG.8

More false positive IgA tTG results were detected in the non-CD controls compared with the IBD controls. A possible explanation may be the presence of IgA anti-BSA antibodies in some of the non-CD control sera, reacting with the BSA used as a blocking agent in some kits.32 However, Lock and colleagues25 did not detect significant IgA anti-BSA antibodies in two disease controls tested, and significant IgA anti-BSA antibodies were not demonstrated in our non-CD controls.

False negative results were found in six of the seven gpl based and five of the six h-tTG based kits in IgA EMA positive

To exclude the possibility that some reactions to tTG were really reactions to blocking agents used in the ELISA kits, anti-BSA and antigelatin antibodies were determined (data not shown). Sera from one IBD control and two patients with CD reacted significantly on the BSA coated ELISA plates, suggesting the presence of IgA anti-BSA antibodies. However, none of the non-CD controls reacted significantly on the BSA coated plates and no sera reacted on the gelatin coated plates.

### DISCUSSION

In this comparison of 13 commercial IgA tTG ELISA kits, we found that the human tTG based kits tested generally demonstrated superior performance (especially specificity) to the gpl-tTG based kits (tables 2,3). However, the use of h-tTG alone was insufficient to confer performance equal to the IgA EMA IIF assay, because only two h-tTG based kits (recombinant h-tTG based Varelisa and purified erythrocyte h-tTG based QUANTA Lite) produced closely comparable results to the IgA EMA IIF assay. Furthermore, two of the gpl-tTG based kits (QUANTA Lite and Eurospital (Trieste, Italy)) had AUC estimations that were not significantly different from the IgA EMA IIF assay. Furthermore, two of the gpl-tTG based kits (QUANTA Lite and Eurospital (Trieste, Italy)) had AUC estimations that were not significantly different from the IgA EMA IIF assay.

Using the manufacturers’ cut off values, false positive results were found in three of the six h-tTG based and six of the seven gpl-tTG based IgA tTG kits in our study (table 4). This has also been reported in other studies.5,9,10,13,20–25,32,33,38 However, as in our study, most of these false positive results were detected in gpl-tTG based ELISAs.5,9,10,13,20–25,32,33,38 These findings raise the important issue of contaminants in gpl-tTG,24,25,38 which may contain other hepatic proteins.26,27 On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gpl-tTG extract (Sigma T5398; Sigma Chemical Co) used in several gpl-tTG based ELISAs5–7,11–13,15,18,20–25,32,33,38 contains multiple bands in addition to the 82–83 kDa tTG band,24,25,38 which only accounted for about 30% of the total protein.29,30 This may be partially overcome by further purification steps, and should be less of an issue with recombinant h-tTG.24,25,38

However, three of the six h-tTG based kits (two recombinant h-tTG based) evaluated also produced false positive results in the absence of IgA EMA and CD, as previously reported.4,14,15 Therefore, other explanations for false positive results are required.

The methods used to extract and purify tissue derived tissue transglutaminase (tTG), produce and process recombinant tTG, and then coat tTG on to ELISA wells may lead to alterations in the tertiary structure of tTG.8

More false positive IgA tTG results were detected in the non-CD controls compared with the IBD controls. A possible explanation may be the presence of IgA anti-BSA antibodies in some of the non-CD control sera, reacting with the BSA used as a blocking agent in some kits.32 However, Lock and colleagues25 did not detect significant IgA anti-BSA antibodies in two disease controls tested, and significant IgA anti-BSA antibodies were not demonstrated in our non-CD controls.

False negative results were found in six of the seven gpl based and five of the six h-tTG based kits in IgA EMA positive

---

### Table 3

<table>
<thead>
<tr>
<th>Manufacturer’s cut off point</th>
<th>ROC plot analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut off</td>
<td>CD (sensitivity)</td>
</tr>
<tr>
<td>IgA EMA IF/Binding Site</td>
<td>1/4 49/49 (100%) 0/34 (100%) 0/30</td>
</tr>
<tr>
<td>Human h-TG based ELISA/Orgentec</td>
<td>15 U/ml 35/49 (71%) 0/34 (100%) 0/30</td>
</tr>
<tr>
<td>Human h-TG based ELISA/QUANTA Lite</td>
<td>10 U/ml 49/49 (100%) 5/34 (85%) 2/30</td>
</tr>
</tbody>
</table>

---

### Table 4

<table>
<thead>
<tr>
<th>Assay type/Manufacturer</th>
<th>Cut off</th>
<th>CD (sensitivity)</th>
<th>Non-CD controls (specificity)</th>
<th>IBD controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig liver tTG</td>
<td>0.406</td>
<td>Eurospital</td>
<td>0.908</td>
<td>0.494</td>
</tr>
<tr>
<td></td>
<td>0.073</td>
<td>0.014*</td>
<td>0.128</td>
<td>Immcx</td>
</tr>
<tr>
<td></td>
<td>0.034*</td>
<td>0.166</td>
<td>0.026* 0.013* Quanta Lite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.900</td>
<td>0.518</td>
<td>0.831 0.044* 0.109 IFR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.711</td>
<td>0.268</td>
<td>0.816 0.115 0.020* 0.616 Medizyme</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.027*</td>
<td>0.093</td>
<td>0.043* 0.008* 0.317 0.051 0.023* Orgentec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.023*</td>
<td>0.058</td>
<td>0.022* 0.006* 0.102 0.027* 0.014* 0.198 Varelisa</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.022*</td>
<td>0.057</td>
<td>0.026* 0.006* 0.123 0.030* 0.016* 0.249 Aesku.lab 0.264</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.056</td>
<td>0.071</td>
<td>0.063 0.007* 0.597 0.067 0.024* 0.195 0.254 Binding Site</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.033*</td>
<td>0.089</td>
<td>0.047* 0.006* 0.427 0.056 0.024* 0.900 0.186 0.301 0.738 Eurospital 0.738</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.023*</td>
<td>0.057</td>
<td>0.023* 0.006* 0.106 0.027* 0.014* 0.195 0.480 0.278 0.194 0.200 Quanta Lite</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05
AUC, area under curve; tTG, tissue transglutaminase.
Take home messages

- In general, the human tissue transglutaminase (h-Tg) based kits tested demonstrated superior performance (especially specifically) to the guinea pig liver Tg (g-Tg) based kits.
- Because this was a general and not a universal funding, factors other than antigen source are important in determining kit performance.
- Most of the kits performed significantly better when the cut off values/decision thresholds were adjusted via receiver operating characteristic plot analysis, which emphasizes the importance of cut off point revalidation by laboratories, using appropriate samples from their referral population.

ACKNOWLEDGEMENTS

We acknowledge K Smithers (Central Sydney Immunology Laboratory, Royal Prince Alfred Hospital) for excellent technical assistance; the Australian distributors of the commercial IgA Tg kits for the generous provision of their kits for assessment in this study; and P Hobson (Department of Immunology, Sullivan Nicolaides Pathology, Brisbane, Australia) for providing some of the sera for the study and performing some of the assays.

Authors’ affiliations

R C W Wong, R J Wilson, Division of Immunology, Queensland Health Pathology Services, Princess Alexandra and Royal Brisbane Hospitals, Brisbane, Australia

R H Steele, South Western Sydney Area Pathology Service, Liverpool Hospital Campus, Sydney, Australia

G Radford-Smith, Department of Gastroenterology, Royal Brisbane Hospital, Brisbane, Australia

S Adelstein, Central Sydney Immunology Laboratory, Royal Prince Alfred Hospital, Sydney, Australia

REFERENCES

New JCP online submission and review system

We are pleased to inform authors and reviewers of the new online submission and review system at JCP. Developed by High-Wire Press (CA, USA), Bench Press is a fully integrated electronic system that utilises the web to allow rapid and efficient submission of manuscripts. It also allows the peer review process to be conducted entirely online. We are one of the first journals in the BMJ Special Journals group to go online in this way. The aim, apart from saving trees, is to speed up the often frustratingly slow process (for both authors and editors) from submission to publication. Many reviewers might appreciate this too. We are very excited with this new development and would encourage authors and reviewers to use the online system whenever possible. As editors, we will use it all the time, the up side being lack of need to travel to the editorial office to deal with papers, the down side having no more excuses to postpone decisions on papers because we are “at a meeting”!

The system is very easy to use and should be a big improvement on the current peer review process. Full instructions can be found on Bench Press http://submit-jcp.bmjjournals.com and JCP online at http://www.jclinpath.com. Please contact Natalie Davies, Project Manager, HYPERLINK mailto:ndavies@bmjgroup.com for any further information.

H Holzel, P van Diest
A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits
R C W Wong, R J Wilson, R H Steele, G Radford-Smith and S Adelstein

doi:

Updated information and services can be found at:
http://jcp.bmj.com/content/55/7/488

These include:
Supplementary Material
Supplementary material can be found at:
http://jcp.bmj.com/content/suppl/2002/09/19/55.7.488.DC1

References
This article cites 44 articles, 6 of which you can access for free at:
http://jcp.bmj.com/content/55/7/488#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Errata
An erratum has been published regarding this article. Please see next page or:
/content/55/10/800.2.full.pdf

Topic Collections
Articles on similar topics can be found in the following collections
- Immunology (including allergy) (1664)
- Clinical diagnostic tests (805)
- Inflammatory bowel disease (40)
- Small intestine (45)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/
Acute erythremic myelosis (true erythroleukaemia): a variant of AML FAB-M6

Our group has been actively researching the acute erythroleukaemias since the late 1980s, during which time, we have developed and extensively published our classification1:5.

- acute erythroleukaemia, M6a (traditional FAB-M6, DiGuglielmo’s syndrome, 1917)
- acute erythroleukaemia, M6b (pure erythroleukaemia, DiGuglielmo’s disease, 1926)
- acute erythroleukaemia, M6c (mixed erythroleukaemia).

We have also published abstracts and presented this classification at meetings of the International Academy of Pathology, International Society of Haematology, and the American Society of Haematology, and our recommendations have also been cited in the recent literature. Therefore, we are surprised to find the M6b subtype now “discovered” and re-named in the article by Hasserjian et al.,6 and the M6c subtype completely overlooked. Although we suspect this incongruity to be an oversight, we believe it is important to set the record straight.

This established classification of the acute erythroleukaemias is based partly on the old FAB criteria and also upon morphological, cytochemical, and immunophenotypic criteria.7 All bone marrow aspirates demonstrate ≥ 50% erythroid precursors, with erythroid dysplasia. Dysplasia of the granulocytic and megakaryocytic cell lines may or may not be present. The M6a subtype is defined as ≥ 30% blasts of the non-erythroidic component (FAB exclusion criteria); the M6b subtype is defined as ≥ 30% promonoblasts of the erythroidic elements; and the M6c subtype has ≥ 30% blasts and ≥ 30% promonoblasts by the aforementioned exclusion criteria. Because the dysplastic changes may, at times, make definitive characterisation of the blasts as erythroidic versus non-erythroidic difficult, the morphological features must always be confirmed by cytochemical stains, immunohistochemical stains, and/or flow cytometric analysis.

These three separate subtypes must be distinguished from one another to provide useful prognostic information for the clinician and the patient. When treated with the standard myeloid protocol, the M6a and M6c subtypes demonstrate a very high remission rate, whereas most patients with the M6b subtype remain refractory to treatment. Notably, patients with the M6c subtype remain in remission for a significantly shorter time than the M6a group. Mean survival for these subtypes is: M6a, 31.4 (SD, 32) months; M6b, 3.13 (SD, 4.2) months; M6c, 10.5 (SD, 12.7) months.

The malignant clonal cell of origin manifesting as acute erythroleukaemia of any subtype appears to be a multipotential stem cell,8 which shows varying degrees of erythroidic and granulocytic lineage maturation. Therefore, the three distinct subtypes of acute erythroleukaemia are not three separate diseases, but rather represent a spectrum of the same disease. The poor remission rate and short survival characteristics of this disorder are dependent upon: (1) a high pronormoblast to myeloblast ratio within diagnostic bone marrow aspirates, (2) a high proliferative index, (3) “unfavourable” cytogenetic aberrations, and (4) a high incidence of P-glycoprotein expression (the multidrug resistance phenotype).

F M Mazzella
Medical College of Georgia, Augusta, 30912-3603, Georgia, USA; fmazzella@mail.mcg.edu

H R Schumacher
University of Cincinnati Medical Centre, Cincinnati, 45367, Ohio, USA

References

Corrections


Detection of the CD56+/CD45– immunophenotype by flow cytometry in neuroendocrine malignancies. Bryson GJ, Lear DJ, Williamson R, et al. J Clin Pathol 2002; 55:335–7. The quotation on page 335 was inadvertently cut out of the first paragraph (it should have remained there as the second sentence) and should begin with CD56 not CD59.