Development of a quantitative immuno-PCR assay and its use to detect mumps-specific IgG in serum

Anne McKie, Dhanraj Samuel, Bernard Cohen, Nicholas A. Saunders*

Virus Reference Division, Central Public Health Laboratory, 61 Colindale Avenue, Colindale, NW9 5HT, London, UK

Received 30 August 2001; received in revised form 16 November 2001; accepted 20 December 2001

Abstract

Determination of the immune status of individuals to vaccine-preventable diseases requires an assay that can detect antibodies that may be present at very low levels, especially when natural or vaccine exposure may have been many years previously. Immuno-PCR (iPCR) has recently been described as an ultrasensitive method for the detection of antigens and we have adapted the method for the quantification of antibodies to mumps virus. The procedure used was similar to an indirect ELISA except that the detecting antibody (anti-human IgG) was chemically conjugated to a short capture oligonucleotide rather than an enzyme. The capture oligonucleotide was then detected by the addition of target DNA, which was designed to hybridise to the capture oligonucleotide and function as a template for real-time PCR. The quantity of target DNA detected by the PCR depended upon the level of specific antibody in the test sample. We found that the sensitivity (and specificity) of the iPCR assay did not exceed that of the conventional ELISA. The sensitivity was limited by nonspecific binding of human IgG to the solid phase. Further development of reagents and assay formats is necessary to fully exploit the potential of quantitative iPCR, so that potential improvements in the sensitivity of anti-mumps IgG detection can be realised. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Immuno-PCR assay; Real-time PCR; Mumps antibody detection

1. Introduction

The use of labelled antibodies in immunoassays offers the potential for developing highly sensitive

Abbreviations: iPCR, immuno-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; MMR, measles, mumps and rubella; tDNA, target DNA; PBS, phosphate buffered saline; PBST, phosphate buffered saline containing Tween 20; SPDP, N-succinimidyl-3-(2-pyridyldithio) propionate; SMCC, succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate; BSA, bovine serum albumin; SD, standard deviation.

* Corresponding author. Tel.: +44-020-8200-4400; fax: +44-020-8200-1569.
E-mail address: nsaunders@phls.org.uk (N.A. Saunders).
clonal antibodies labelled with a chemiluminescent acridium ester was shown to be 50 photon counts per attomole of label (Weeks et al., 1984). Similarly, the use of europium labels in time-resolved fluorescence has permitted the development of sensitive immunoassays by reducing background noise and consequently increasing signal-to-noise ratios (Jackson and Ekins, 1986).

Enzymes such as horseradish peroxidase and alkaline phosphatase have also been widely used as labels. They have the potential for signal amplification since one molecule of enzyme may catalyse the conversion of many substrate molecules to detectable product. In practice, however, the colorimetric substrates used to monitor enzyme activity limit the sensitivity with which enzyme labels can be detected since relatively large concentrations of chromogens need to be generated for spectrophotometric detection. Enzyme assays have therefore not usually demonstrated much greater sensitivity than radioisotopic methods, though the use of fluorogenic and radioactive substrates or enzyme amplification methods have increased detection limits (Harris et al., 1979; Stanley et al., 1985; Johannsson et al., 1986; Ruan et al., 1993; Zhang et al., 2001).

Recently, DNA labels that are detected using PCR have been used in the development of ultrasensitive immuno-PCR (iPCR) assays (Sano et al., 1992; Ruzicka et al., 1993; Zhou et al., 1993; Hendrickson et al., 1995; Maia et al., 1995; Case et al., 1997; Wu et al., 2001). Sano et al. (1992) reasoned that since single target nucleic acid molecules can potentially be detected by PCR, the use of DNA labels would permit highly sensitive assays. Using this approach they were able to detect as few as 580 molecules of immobilised bovine serum albumin. To date, iPCR has been used for the sensitive detection of a variety of soluble protein molecules such as cytokines (Sanna et al., 1995; Saito et al., 1999), tumour markers (Suzuki et al., 1995) and hormones (Hendrickson et al., 1995). However, it has so far not been applied to the detection of antibodies to human pathogens. In this study we describe the development of a quantitative iPCR assay and assess its potential for the detection of antibody to mumps virus. Although the incidence of mumps in the United Kingdom has fallen to low levels since the introduction of measles, mumps and rubella (MMR) vaccination, serological surveillance remains important to provide early warning of any accumulation of susceptible individuals that could facilitate a resurgence of disease (Osborne et al., 2000).

2. Materials and methods

2.1. iPCR format

A schematic diagram of the iPCR assay developed for the detection of antibodies to mumps virus is shown in Fig. 1. First, serum samples were incubated in wells coated with recombinant mumps nucleoprotein antigen. After washing, wells were incubated with a mixture of conjugate (anti-human IgG covalently coupled to capture oligonucleotide) and target DNA (tDNA), which was designed to hybridise to capture oligonucleotide. After washing, hybridised tDNA was released into solution by restriction enzyme digestion and subsequently quantified by real-time PCR using the LightCycler™. The amount of tDNA released from the conjugate was proportional to the level of specific mumps IgG antibody in the test sample.

2.2. Design of target DNA, primers and probes

All oligonucleotides were synthesised by MWG-Biotech, UK (Milton Keynes, England) and are shown in Table 1. A single-stranded (ss) amplification target oligonucleotide (tDNA), 89 base pairs in length, was designed with six defined sequence regions: complementary sequence to the capture oligonucleotide; Hind III restriction site; 5′ primer sequence; complementary sequence to the 3′ primer; and an internal probe sequence. Two primers (Table 1) of 20 base pairs in length were used to amplify a 67-bp region of the tDNA. They had a G+C content of 45–50%, similar melting temperatures and no complementary sequences. Two additional oligonucleotides were used. The internal probe was designed to hybridise to a sequence between the primer sites and was used to detect specific PCR product during amplification. It was labelled with Cy5 at the 5′ terminal and blocked with biotin at the 3′ terminal to prevent it acting as a primer during PCR. The capture oligonucleotide was complementary to the 5′ end of the tDNA and contained a Hind III restriction digest site to enable the release of
hybridised tDNA into solution prior to DNA quantification. In the assay development, the PCR was optimised first, followed by the antibody detection system, as described below.

2.3. Quantitative PCR using the LightCycler™

Five DNA standards, containing between $10^4$ and $10^8$ copies of tDNA, were tested in parallel with the samples to allow accurate quantification. The standards were prepared by making serial ten fold dilutions of tDNA of known concentration in nuclease-free water (Promega UK, Southampton, UK) containing 5 µg/ml of herring sperm DNA. In addition, at least one negative control containing no tDNA was included in each batch of PCR tests. PCR reactions were carried out using the LightCycler™ (Bio/Gene, Kimbolton, England) in a final volume of 10 µl containing 50 mM Tris–HCl, pH 8.3; 0.5 mM bovine serum albumin; 3 mM MgCl$_2$; 200 mM deoxynucleoside triphosphates (Gibco, Paisley, Scotland); 0.4 units Platinum Taq DNA polymerase (Gibco); 1/10,000 SYBR Green I (Bio/Gene); 0.5 µM each primer; 0.25 µM internal probe; and 1 µl of tDNA or H$_2$O. The thermal conditions were an initial denaturation at 93 °C for 15 s, followed by 50 amplification cycles of four steps: 93 °C for 0 s; 55 °C for 0 s; 58 °C for 1 s; and 74 °C for 2 s. Fluorescence was measured once per cycle after the 58 °C step. Maximum ramp rates (20 °C/s) were used except for the transition from 55 to 58 °C when the ramp rate was 3 °C/s. Immediately after amplifi-
cation, a two-step melting programme followed: 95 °C for 0 s; and 45 to 95 °C at a ramp rate of 0.2 °C/s with continuous fluorescent monitoring.

2.4. Capture and release of tDNA

Target DNA (3 × 10^12 tDNA copies/μl) was incubated for 10 min at 50 °C, in the presence and absence of biotinylated capture oligonucleotide. Each mixture was diluted 1/100 (final concentration of tDNA approximately 1.5 × 10^10 copies/μl) with phosphate buffered saline (PBS) and 40 μl was added to streptavidin-coated (Thermo Labsystems Oy, Helsinki, Finland) or negative control wells. After removal of unbound material by washing with PBS containing 0.05% Tween 20 (PBST), the well contents were incubated with 40 μl of buffer containing 1 U Hind III, or mock digested, for 1 h at 37 °C. Eluted material was collected and 1 μl used as template for quantitative PCR using the LightCycler™.

2.5. Conjugation of anti-human IgG to capture oligonucleotide

Amino-modified capture oligonucleotide (Table 1) was chemically conjugated to affinity-purified rabbit anti-human IgG (Chemicon International, Temecula, CA) using the heterobifunctional cross linkers N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) by MicroImmune (Brentford, UK).

2.6. Preparation of human IgG- and bovine serum albumin-coated plates

To optimise and evaluate the binding of conjugate (anti-human IgG-capture oligonucleotide) to human IgG, microtitre plates were coated with human IgG or bovine serum albumin (BSA) as a control. Microtitre plate wells (MaxiSorb, Nunc, Life Technologies, Paisley, United Kingdom) were coated with 100 μl of 2 μg/ml affinity purified human IgG (Chemicon) or BSA in 0.05 M sodium carbonate–bicarbonate buffer (pH 9.6) and incubated at 37 °C overnight. After washing with PBS, nonspecific protein binding sites were blocked with 150 μl of 5% SoluPro (Dynagel, Calumet City, IL) in deionised water for 2 h at 37 °C. The wells were then washed, left to dry overnight at 37 °C and stored in a sealed container until use.

2.7. Serum samples

Mumps-specific IgG negative, weak positive and strong positive sera (as determined with a commercial ELISA Kit; Behring Enzygnost; Behringwerke, Marburg, Germany) were used to optimise the iPCR assay and subsequently used as controls in further experiments. A panel of 88 additional sera, which had been submitted for diagnostic testing by the same kit, was used to evaluate the iPCR assay.

2.8. Mumps-specific IgG iPCR

After experiments to optimise the assay conditions, the iPCR assay was performed as follows:

2.8.1. Coating

Microtitre plate wells were coated with 100 μl of 2 μg/ml recombinant mumps nucleoprotein antigen (Microimmune) in PBS containing 0.08% sodium azide, pH 7.2 and incubated at 37 °C overnight. After washing with PBS, the wells were blocked with SoluPro (Dynagel), dried and stored as described above.

2.8.2. Antibody capture

Sera were diluted 1/200 using assay diluent (PBS containing 10% normal goat serum, 2% skimmed milk, and 0.1% Tween 20). Twenty microlitres was added to wells and incubated at 37 °C for 30 min. The wells were then washed 12 times with PBST.

2.8.3. Detection of captured antibody

Conjugate/tDNA mixture was prepared by adding 1 μl of conjugate (anti-human IgG-capture oligonucleotide) and 50 μl of tDNA (10^10 copies/μl) to 1 ml of assay diluent. Twenty microlitres were added to the wells and incubated at 37 °C for 1 h. Wells were washed 12 times with PBST and then digested with 1 U Hind III (New England Biolabs (UK), Hitchin, Hertfordshire) for 1 h at 37 °C. Eluted material was collected and 1 μl was used as template for quantitative PCR using the LightCycler™. Results were quantified in terms of copies of amplified tDNA per microliter eluted from each well. All wash steps
were performed using an automated plate washer (Labsystems Wellwash 4 Mk 2).

2.9. Statistical methods

2.9.1. iPCR assay cut-off

A positive iPCR result was defined as any value greater than two standard deviations (SD) above the mean value of the negative control, which was tested in parallel with samples. The SD was determined by the analysis of a panel of 17 sera negative for mumps specific IgG by the Behring Enzygnost ELISA.

2.9.2. Reproducibility of iPCR assay

The reproducibility data were analysed in the BMDP program 8 V, which performs general mixed model analysis of variance for equal cell sizes (Dixon, 1992). The data were analysed for each serum sample separately to obtain serum specific estimates. In the analysis wells and PCRs were declared to be random effects.

3. Results

3.1. Sensitivity of the PCR using the LightCycler™

Under optimised amplification conditions, the PCR was able to detect $10^2$ copies of tDNA/µl, with a wide dynamic range of >7 logs. Melting peak analysis revealed peaks at 63 and 83 °C, corresponding to the melting temperature of the internal probe/tDNA duplex and the full-length product, respectively (data not shown).

3.2. Capture and release of tDNA

Experiments were performed to determine whether the tDNA would hybridise to the capture oligonucleotide, and to determine the optimal conditions for the release of hybridised tDNA prior to PCR. Binding of tDNA to streptavidin-coated (Strep+) and uncoated (Strep−) wells, in the presence or absence of biotinylated capture oligonucleotide, was investigated. We found tDNA bound at very low concentrations to Strep− wells, both in the presence and absence of capture oligonucleotide ($1 \times 10^4$ copies tDNA/µl out of $1.5 \times 10^{10}$ copies tDNA/µl added bound to the wells).

A similar level of binding was observed when tDNA was added to Strep+ wells in the absence of capture oligonucleotide. In contrast, in the presence of streptavidin and capture oligonucleotide, high levels of tDNA ($1 \times 10^7$ copies tDNA/µl) were detected. This suggested that the tDNA was binding to the streptavidin-coated wells via the biotinylated capture oligonucleotide. The optimal release of hybridised tDNA from Strep+ wells was achieved by restriction digestion ($1 \times 10^6$ copies tDNA/µl detected), however, the tDNA could also be eluted simply by incubating wells with buffer for a similar time period ($3 \times 10^8$ copies tDNA/µl detected).

3.3. Binding of anti-human IgG-capture oligonucleotide conjugate to human IgG

The anti-human IgG-capture oligonucleotide conjugate was initially evaluated with respect to its capacity to bind to immobilised human IgG and to BSA as a control. As shown in Table 2, when a constant amount of tDNA was incubated with tenfold dilutions of conjugate, very low levels of tDNA were detected in the BSA-coated wells compared with the human IgG wells. The dilution of conjugate, which

<table>
<thead>
<tr>
<th>Conjugate dilution factor</th>
<th>tDNA (copies/well)</th>
<th>BSA-coated wells (tDNA/µl)</th>
<th>Human IgG-coated wells (tDNA/µl)</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^1$</td>
<td>$10^1$</td>
<td>$1.9 \times 10^5$</td>
<td>$1.0 \times 10^8$</td>
<td>526</td>
</tr>
<tr>
<td>$10^2$</td>
<td>$10^1$</td>
<td>$4.9 \times 10^4$</td>
<td>$1.0 \times 10^8$</td>
<td>2041</td>
</tr>
<tr>
<td>$10^3$</td>
<td>$10^1$</td>
<td>$1.3 \times 10^4$</td>
<td>$4.6 \times 10^7$</td>
<td>3538</td>
</tr>
<tr>
<td>$10^4$</td>
<td>$10^1$</td>
<td>$1.4 \times 10^4$</td>
<td>$5.1 \times 10^6$</td>
<td>364</td>
</tr>
<tr>
<td>$10^5$</td>
<td>$10^1$</td>
<td>$2.1 \times 10^3$</td>
<td>$1.2 \times 10^6$</td>
<td>571</td>
</tr>
<tr>
<td>–</td>
<td>$10^1$</td>
<td>$1.4 \times 10^4$</td>
<td>$6.1 \times 10^5$</td>
<td>4</td>
</tr>
<tr>
<td>$10^1$</td>
<td>–</td>
<td>$1.8 \times 10^3$</td>
<td>$3.4 \times 10^4$</td>
<td>19</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>$3.2 \times 10^{-1}$</td>
<td>$2.9 \times 10^1$</td>
<td>91</td>
</tr>
</tbody>
</table>

Serial tenfold dilutions of conjugate were prepared in conjugate diluent (2% BSA; 0.05% Tween 20 in PBS) and 40 µl was added to microtitre wells coated with human IgG and BSA, where BSA served as a control for nonspecific binding. The wells were incubated for 1 h at 37 °C, washed, then incubated for 1 h at 37 °C with 40 µl of diluted tDNA ($10^1$ copies/well). After washing, tDNA was eluted and quantified using the LightCycler™.

S/N denotes signal to noise ratio, which is the amount of tDNA eluted from the human IgG-coated wells divided by the amount eluted from the BSA-coated wells.

(−) denotes no conjugate or tDNA was added to the wells.
resulted in the highest signal-to-noise ratio, was 1/1000, hence this was used in subsequent experiments. At a conjugate dilution of 1/10,000, the signal obtained was approximately tenfold lower, but the level of nonspecific binding remained the same. In wells that received tDNA but no conjugate, only background levels of tDNA were detected. There was evidence of cross-contamination between wells, as traces of tDNA were detected in wells to which none had originally been added. A similar experimental approach was used to determine the optimal amount of tDNA for use in the iPCR assay, where the conjugate was fixed at a dilution of 1/1000 and the tDNA was varied. The optimal amount was found to be $10^{10}$ copies/well.

3.4. Reproducibility of the iPCR assay

The reproducibility of the iPCR assay was investigated by adding control sera to different wells on the same microtitre plate in quadruplicate. tDNA from each well was then quantified in four separate PCR assays. Table 3 shows the mean values ($\log_{10}$ scale) and standard deviations obtained. Both the inter-PCR and inter-well standard deviations were low, indicating that the assay was highly reproducible.

3.5. Evaluation of the iPCR assay

The iPCR assay for mumps-specific IgG was evaluated in its optimised format using 88 serum samples previously tested in the commercial ELISA. Concordant results were obtained for 84 sera. Of the remaining four, one specimen was ELISA-positive and iPCR-negative, one was ELISA-negative and iPCR-positive, and two samples with equivocal ELISA results were iPCR-negative. For three of these discrepancies the ELISA result was very close to the cutoff (Table 4). Compared to the ELISA, the iPCR was 98.6% (71/72) sensitive and 92.9% (13/14) specific. When the ELISA absorbance readings were plotted against the iPCR results for the 88 sera tested, there was good correlation (correlation coefficient 0.88) between the values (Fig. 2).

Table 3
Reproducibility of the iPCR assay

<table>
<thead>
<tr>
<th>Control sera</th>
<th>Mean ($\log_{10}$)</th>
<th>SD (wells)$^a$</th>
<th>SD (PCR)$^b$</th>
<th>SD (all)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>4.86</td>
<td>0.08</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Weak positive</td>
<td>6.09</td>
<td>0.04</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Strong positive</td>
<td>6.69</td>
<td>0.07</td>
<td>0.10</td>
<td>0.13</td>
</tr>
</tbody>
</table>

$^a$ SD (standard deviation) for the averaged measurements from different wells.

$^b$ SD for the averaged measurements of different PCRs from the same well.

$^c$ SD obtained for all the measurements.

Table 4
Data on discrepant samples

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Age (years)</th>
<th>Clinical details</th>
<th>Mumps-specific IgM$^a$</th>
<th>Mumps-specific IgG (OD)$^b$</th>
<th>iPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6</td>
<td>no information</td>
<td>negative</td>
<td>equivocal (0.106)</td>
<td>negative</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>hospital staff immunity check</td>
<td>not tested</td>
<td>positive (0.213)</td>
<td>negative</td>
</tr>
<tr>
<td>31</td>
<td>32</td>
<td>acute parotid swelling</td>
<td>negative</td>
<td>negative (0.041)</td>
<td>positive</td>
</tr>
<tr>
<td>73</td>
<td>37</td>
<td>? recent mumps</td>
<td>negative</td>
<td>equivocal (0.102)</td>
<td>negative</td>
</tr>
</tbody>
</table>

$^a$ Mumps-specific IgM antibody capture radioimmunoassay (Perry et al., 1993).

$^b$ Behring Enzygnost ELISA (negative = < 0.1; equivocal=0.1 – 0.2; and positive = > 0.2).
4. Discussion

The detection of mumps-specific IgG plays an important role in immunity surveillance, monitoring the efficacy of vaccination programmes, identifying susceptible cohorts in the population and shaping future vaccination policies. Immunoassays are the method of choice for viral antibody detection, but the limited sensitivity of current mumps-specific IgG assays has restricted the value of the procedure (Pipkin et al., 1999). This poses a particular problem when measuring mumps-specific IgG prevalence in populations in which mumps virus infection has been controlled by MMR vaccination. Firstly, antibody responses to vaccination are lower than following infection with wild type virus and, secondly, levels of antibody in the population are no longer boosted by continuing circulation of the virus. In a recent ELISA-based study of pre-school children in the United Kingdom, 15% were mumps antibody negative, compared to 8% and 10% for measles and rubella, respectively (Gay et al., 1997). It is unclear whether this was due to the mumps test being less sensitive or to the mumps virus component of the vaccine being less antigenic than the other two components.

To improve assay sensitivity, we developed an indirect, quantitative iPCR assay for the detection of mumps-specific IgG in serum. The distinctive features of our assay were: (a) the conjugate, which had the dual function of binding to any immobilised human IgG and capturing tDNA; (b) the use of restriction enzyme digestion for the specific release of hybridised tDNA; and (c) the quantification of tDNA by real-time PCR using the LightCycler™.

Several versions of iPCR that deliver improvements in assay sensitivity have been published. These methods differ mainly in the way target DNA is linked to detecting antibody (Sano et al., 1992; Ruzicka et al., 1993; Zhou et al., 1993; Hendrickson et al., 1995). Sano et al. (1992) used a recombinant chimeric protein that contained two moieties, protein A and streptavidin, which bound to the Fc fragment of antibodies and biotin-modified DNA, respectively. However, the lack of availability and limited application of the chimera led others to use one of the biotin-binding proteins, avidin or streptavidin, to link biotinylated DNA and biotinylated antibody (Ruzicka et al., 1993; Zhou et al., 1993). More recently, directly labelled conjugates have been described where DNA is covalently coupled to detecting antibody (Hendrickson et al., 1995; Joerger et al., 1995). The use of such conjugates permits simple assay formats to be adopted since fewer washing and incubation steps are required. In addition, multiple analytes can be simultaneously detected.

In this study, a novel approach was used to link detecting antibody to target DNA. Secondary antibody was covalently conjugated to a short oligonucleotide, which was partly complementary to the 5' end of tDNA. Restriction digestion was then used to release hybridised tDNA from the solid phase, though an alternative way of releasing tDNA would have been to use heat treatment (Sanna et al., 1995). Initially, we reasoned that restriction digestion would improve the specificity of the test; however, our experiments indicated that the release of tDNA could be achieved simply by incubating wells with restriction digestion buffer at 37°C. The nature of this release is unclear; the buffer would not be expected to denature the tDNA from the capture oligonucleotide, but it may be that the antigen or antibodies desorb from the solid phase over time.

Amplification on the LightCycler™ proved very useful in the development of the assay by providing rapid and reproducible quantification of tDNA. Once tDNA samples were ready for PCR, amplification and analysis were completed within 30 min, considerably faster than by conventional PCR. Quantitative PCR was essential to allow the relative levels of nonspecific binding of tDNA, conjugate and human IgG to be accurately determined. In previous studies using conventional PCR, based on the detection of amplicons after gel electrophoresis, it was not possible to determine accurately the contribution of the specific signal compared to the nonspecific signal. We found that nonspecific binding of human IgG was the main limiting factor affecting the sensitivity of the assay, compromising the advantages gained by PCR amplification. Hence, it was necessary to define the experimental results on the basis of signal-to-noise ratios rather than the presence or absence of specific product.

To minimise nonspecific binding extensive optimisation experiments were performed. Most critical were the working concentrations of conjugate and tDNA. We also found that signal-to-noise ratios improved
when milk was used as a blocking agent in the assay diluent. This is consistent with the results of a study investigating the efficacy of different blocking agents for suppressing background caused by nonspecific protein adsorption to the solid phase (Vogt et al., 1987). To minimise the level of nonspecific binding of target DNA, sheared sperm DNA has been used by several groups (Sano et al., 1992; Maia et al., 1995; Sperl et al., 1995; Kakizaki et al., 1996). However, addition of sheared sperm DNA to the assay diluent did not improve the performance characteristics of our assay. As reported by others (Sano et al., 1992; Kakizaki et al., 1996) and confirmed in the present study (data not shown), extensive washing of wells with detergent-containing buffer to remove unbound reagents was essential to minimise background noise.

When the optimised iPCR was compared with the ELISA, concordance was good. Discrepant findings were obtained in four cases, two of which were attributable to equivocal values in the commercial ELISA. A possible explanation was the use in the two assays of different antigen preparations for capturing the anti-mumps antibody. We employed a recombinant mumps nucleoprotein antigen in contrast to the whole virus preparation containing a mixture of native antigens used in the Behring Enzygnost ELISA. The use of a single purified antigen has the potential to give fewer false-positive results, but may contribute to false-negative results since antibodies to other mumps antigens will not be detected. In the clinical setting, a false-negative result is relatively less important than a false-positive result, as in the latter case the individual would not be vaccinated, leaving them susceptible to wild-type mumps infection.

The present report demonstrates the feasibility of using iPCR for the detection of antibodies in human serum. In contrast to previously published reports, we were unable to show an enhanced sensitivity over the ELISA but we have shown (in a way that was not possible in previous studies based on qualitative PCR endpoints) that the main limitation in iPCR is nonspecific binding of assay components to the solid phase. Also, the wide dynamic range of the assay gives a more accurate assessment of high level antibody responses and may therefore be useful in longitudinal studies.

Improvements in reagents and assay formats, leading to enhanced performance characteristics, will be required before iPCR can be routinely used for diagnosis of infection or determination of immune status. In addition, a solid phase more compatible with antigen coating, automated washing and quantitative PCR would greatly simplify and advance the applications of iPCR.

**Acknowledgements**

We would like to thank the Immunisation and Diagnosis Unit for their laboratory support; Pauline Rogers of the PHLS Statistics Unit for statistical advice and analysis of the data; and Philip Mortimer for reviewing the manuscript.

**References**


