Comparison of Creatinine As Determined with the Ames Seralyzer and by Three Jaffé-Based Methods

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We compared results for urinary creatinine, serum creatinine, and creatinine clearance, as determined with the Ames Seralyzer, with results determined with the Beckman Astra, the DuPont aca, and Technicon's AutoAnalyzer and SMAC. Results for urinary creatinine from the Seralyzer differed significantly (p < 0.05) from those obtained with the Astra and AutoAnalyzer, but not with the aca. The Seralyzer results for serum creatinine were at least 1.0 mg/L higher (p < 0.05) than by the other three methods. Results for creatinine clearance from the Seralyzer were 8 to 11 mL/min lower (p < 0.05) than results by the other three methods. These differences are related to the positive interference by bilirubin in the Seralyzer creatinine method. We also evaluated 23 other compounds for interference with these methods for creatinine.

Additional Keyphrases: urine · intermethod comparison · variation, source of · creatinine clearance · reflectance photometry · AutoAnalyzer, aca, SMAC, Astra compared

In assaying creatinine with the Ames Seralyzer, a multipoint kinetic method, a dry reagent pad, and diffuse reflectance photometry are used (1, 2). The basis of the method differs from most others, involving the chromogen 3,5-dinitrobenzoate (instead of alkaline picrate) and a different technique of photometry.

We compared results for serum creatinine, urinary creatinine, and creatinine clearance as determined with the Seralyzer with results by three other commonly used methods: Technicon's continuous-flow (SMAC for serum and AutoAnalyzer for urine, because the SMAC does not measure urinary creatinine), DuPont's aca, and Beckman's Astra. Because the Seralyzer requires a hand-pipetting step, we also evaluated the variation introduced by different operators at this point. To test the effect of potentially interfering compounds on a reagent not so thoroughly studied as alkaline picrate, we evaluated 23 compounds and bilirubin for interference with the Seralyzer method vs their interference with the aca and Astra methods.

Materials and Methods

Comparison Study

Apparatus: The Seralyzer reflectance photometer (Ames Division, Miles Laboratories, Inc., Elkhart, IN 46516) measures reflected light at 560 nm as percent reflectance (%R), analogous to measuring transmittance in absorbance photometry (2). The reflectance, though not linearly related to concentration, is converted to a linear function of concentration at a specified wavelength by the Kubelka-Munk equation: F(R) = (1 - R)²/2R ~ aSc or the log F(R) = log c + D, where F(R) is the function of the reflectance, e is the molar absorptivity at the specified wavelength, c is the concentration of analyte, S is the scattering constant, and D is a constant (3). The reagent strip is placed on a holder, 30 µL of sample is pipetted onto the reagent pad, the start button is pressed, and the holder and strip are inserted into the instrument. The Seralyzer measures reflectance after an incubation period of 30 s and calculates the rate of reaction and the creatinine concentration on the basis of a calibration with high- and low-concentration standards supplied by Ames.

The instruments we used in our comparison were the AutoAnalyzer II and SMAC (Technicon Instruments Corp., Tarrytown, NY 10591), the Astra (Beckman Instruments, Inc., Brea, CA 92824), and the aca (DuPont Instruments, Wilmington, DE 19888).

Procedure: We selected paired urine and serum specimens with creatinine clearance values evenly distributed throughout the range of 20 to 120 mL/min, as calculated from values determined with the Technicon instruments. We determined serum and urine creatinine with the aca, the Astra, and the Seralyzer, according to the manufacturer's instructions. We diluted urine 10-fold with DuPont enzyme diluent (an albumin solution) for samples determined by the aca and Astra, or with water for the Seralyzer assays (according to the manufacturer's instructions). From these results we calculated creatinine clearance for each paired urine and serum specimen. The Technicon systems were arbitrarily chosen as a reference. [This Seralyzer creatinine product insert does not indicate that urine may be used, but only undiluted serum or plasma. For this evaluation, urine specimens were studied for investigational use only.] To evaluate variability among technologists operating the Seralyzer, we performed duplicate determinations of creatinine, designating them as Seralyzer 1 (three technologists) and Seralyzer 2 (one technologist).

Statistical Analysis

We analyzed the data in each category (serum, urine, and clearance) by two-way analysis of variance. If the analysis of variance indicated significant differences among the groups, then Tukey's test was used to identify which groups were different (4). To quantify differences among methods, we analyzed the data by de-biased linear regression, using the Technicon results as the abscissa and run-to-run variance for each method derived from the coefficient of variation as given below in the Precision section (5). We determined total bilirubin concentration with the SMAC for most serum samples for which the difference between creatinine as determined with the Seralyzer and creatinine as determined with the SMAC was >1 mg/L (positive) or <1 mg/L (negative), and we compared the means by Student's t-test.

Interference Study

Reagents: We added the following compounds to pooled serum: cefoxitin (Merck, Sharp & Dohme), cephalin, cephalxin, cephalothin, oxaloacetate, acetate, acetoacetate, pyruvate, cephaloridine, ascorbate (Sigma Chemical Co.), acetohexa-
mide, cefamandole, moxalactam (El Lilly & Co.), acetone (Aldrich Chemical Co.), phenacemide (United States Pharmacopeia), cefazolin (SmithKline & French), cefotaxime (Hoechst-Roussel Pharmaceuticals), α-hydroxybutyrate, fructose, and lactate (Sigma). The highest concentration of added compounds in serum was 1 g/L except for cefoxitin, cephalotin, cephalexin (2 mg/L), phenacemide (300 mg/L), glucose (10 g/L), and bilirubin (126 mg/L). We evaluated the interference caused by bilirubin with a bilirubin reference material (Sigma, lot 14F-6102, 95 mg/L assayed value for total bilirubin in human albumin solution) and protein with a protein diluent (Lancer Microprotein Rapid Stat standard, lot no. 8240, 99 g/L human albumin; Lancer Division, Sherwin Medical, St. Louis, MO 63103). We used NBS Standard Reference Material creatinine, SRM no. 914 (National Bureau of Standards, Washington, DC 20234), for all aqueous solutions of creatinine, and Human Serum SRM no. 909 with an NBS-assigned value of 17.2 mg/L for creatinine for assessing accuracy.

Procedure: We sequentially diluted these solutions with serum or aqueous creatinine, as appropriate, and determined creatinine in duplicate with the Seralyzer and singly with the aca and Astra, analyzing the results by conventional linear regression (creatinine as the dependent variable and the concentration of added compound as the independent variable), the degree of interference being equal to the slope of the regression. N ranged from 7 to 12 for the Seralyzer and 4 to 6 for the Astra and aca. We did not measure interference with the Technicon instruments, because previous studies have shown little interference with them by drugs (6).

With the Seralyzer, we determined creatinine 10 times in the bilirubin reference material. Then, we diluted the bilirubin reference material with an equal volume of water or an equivalent mixture of chloroform and ethanol and determined creatinine with the Seralyzer.

We diluted the protein diluent with distilled, de-ionized water, to give concentrations of 99, 46.5, and 18.6 g of protein per liter, and determined apparent creatinine with the Seralyzer.

Analytical Variables

Linearity. We dissolved creatinine in distilled, de-ionized water, diluted it with water to achieve concentrations of 100, 50, 33, 20, and 10 mg/L, and determined creatinine with the Seralyzer.

Precision. We used high and low controls as provided by the manufacturer for the Seralyzer and Ortho QCS normal and abnormal controls, unassayed (Ortho Diagnostic Systems, Raritan, NJ 08869) for the SMC, Astra, and aca. For urinary creatinine, we used a frozen urine pool (prepared in house). To calculate the variation of creatinine clearance, we used the formula CVc = √(CVc,2 + CVs,2), where CV means coefficient of variation and the subscripts cl, s, and u, mean creatinine clearance, serum creatinine, and urinary creatinine, respectively.

Accuracy. We determined creatinine in a reference sample, Human Serum 909, and the Seralyzer calibration materials (assigned values of 5 and 63 mg/L creatinine) with all instruments, and the concentrations of total bilirubin in the Seralyzer calibration materials with the SMC.

Results

Comparison Study

The results between Seralyzer 1 and 2 showed no significant differences (Table 1) for urinary and serum creatinine and creatinine clearance. There was a significant difference between results from the Seralyzer and the other three analytical systems for serum and urinary creatinine and creatinine clearance, except for urinary creatinine determined with the aca (Table 1). The results from the AutoAnalyzer and aca showed no significant difference for clearance and serum creatinine, while the results from the Astra differed significantly from the other systems for serum and urinary creatinine, but not for creatinine clearance.

The slopes for clearance results from the aca and Astra were within 5% of the AutoAnalyzer slope, while the slope for the Seralyzer (Seralyzer 1) was 15% lower than the AutoAnalyzer slope (Figure 1). For serum, the slopes for the Seralyzer and aca were <5% different, while the slope for the Astra was 7% less than that for the SMC. For urine, the slopes for all instruments were below the slope for the AutoAnalyzer, 4.0% for the Seralyzer, 5.5% for the aca, and 8.5% for the Astra. The slope and y-intercept values for Seralyzer 2 were almost identical to those for Seralyzer 1.

Interference Study

All plots of creatinine vs concentration of compound had correlation coefficients >.85 except for five regressions with small slopes. Concentrations of measured creatinine in pooled serum varied from 9 to 16 mg/L, and y-intercepts varied less than 2 mg/L from the unspiked serum value.

We saw no interference for the determination of creatinine with the Seralyzer for acetaminophen, urea, benzylpenicillin, cefotaxime, beta-hydroxybutyrate, glucose, fructose, lactate, or phenacemide. We saw interference for the determination of creatinine with the Seralyzer for cefoxitin, cephalothin, cephaloridine, acetohexamide, bilirubin, oxalacetate, pyruvate, acetone, acetacetate, cefamandole, cefazolin, cephalexin, cephrapin, moxalastam, and ascorbate (Table 2).

Using one concentration of bilirubin reference material (95 mg/L), we obtained an average positive interference of 0.15 mg of creatinine per milligram of bilirubin (14.6 mg of apparent creatinine per liter per 95 mg of bilirubin per liter, n = 10, SD = 0.013). The diluted samples of bilirubin reference material showed interference of 5.8 ± 0.8 mg creatinine (mean ± SD) for water and 8.8 ± 0.8 mg creatinine for chloroform/ethanol (n = 5 for both).

We found 34 results where the Seralyzer serum creatinine was more than 1 mg/L greater than the SMC creatinine (positive group); this group had a mean total bilirubin concentration of 13 mg/L. For results where Seralyzer creatinine minus the SMC creatinine were ≤1 mg/L, we had results for total bilirubin in 54, with a mean of 5 mg/L. The means for the two groups differed significantly by Student's t-test (p < .01). In the positive group, 21 patients had a total bilirubin concentration >5 mg/L, with a mean total bilirubin concentration of 18.9 mg/L. The mean creatinine concentration in these 21 samples was 15.4 mg/L as determined with the Seralyzer, 13.0 mg/L as determined with the SMC.
Fig. 1. Comparison of instruments by de-biased linear regression analysis with Technicon methods as x-axes.

Instruments (y-axes) are Seralyzer (A-C), Acca (D-F), and ASTRA (G-I). Types of tests are serum creatinine (A, D, G), urinary creatinine (B, E, H), and creatinine clearance (C, F, I). The regression equation, regression line (solid line), line of identity (dashed line), and $S_{xy}$ are shown on each graph. $N$ equals 107, though not all points are shown.

Table 2. Interference Data

<table>
<thead>
<tr>
<th>Compound</th>
<th>Seralyzer</th>
<th>Acca</th>
<th>[Compound]$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoxacetate</td>
<td>0.175(0.71)</td>
<td>3.20(0.99)</td>
<td>300 (10)</td>
</tr>
<tr>
<td>Acetoheamidc $^c$</td>
<td>10.1(0.99)</td>
<td>13.0(0.99)</td>
<td>40-130 (17)</td>
</tr>
<tr>
<td>Acetone $^d$</td>
<td>1.13(0.98)</td>
<td>0.850(0.99)</td>
<td>175 (10)</td>
</tr>
<tr>
<td>Ascorbate $^c$</td>
<td>-0.100(-0.58)</td>
<td>0.650(1.0)</td>
<td>6-20 (12)</td>
</tr>
<tr>
<td>Bilirubin $^c$</td>
<td>9.39(0.98)</td>
<td>-0.248(-0.111)</td>
<td>50-200</td>
</tr>
<tr>
<td>Oxaloacetate</td>
<td>5.80(0.99)</td>
<td>2.80(0.99)</td>
<td>3.10(0.99)</td>
</tr>
<tr>
<td>Pyruvate $^d$</td>
<td>8.50(0.99)</td>
<td>4.35(0.99)</td>
<td>5.05(0.99)</td>
</tr>
<tr>
<td>Cepha antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefamandol $^c$</td>
<td>0.825(0.97)</td>
<td>0</td>
<td>133-533 (13)</td>
</tr>
<tr>
<td>Cefazolin $^e$</td>
<td>0.636(0.99)</td>
<td>0.122(0.85)</td>
<td>39-76 (14)</td>
</tr>
<tr>
<td>Cefoxitin $^e$</td>
<td>4.63(0.99)</td>
<td>3.67(0.99)</td>
<td>223 (15)</td>
</tr>
<tr>
<td>Cephalexin $^e$</td>
<td>0.120(0.72)</td>
<td>0.051(0.88)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>1.53(0.95)</td>
<td>1.50(1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Cephalpin</td>
<td>0.373(0.67)</td>
<td>0.040(0.68)</td>
<td>103(8)</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>1.01(0.96)</td>
<td>2.89(0.99)</td>
<td>0</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>0.336(0.85)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$Units are mg of apparent creatinine per liter per milligram of compound per liter. Correlation coefficients given in parentheses.

$^b$Concentration of compound expected from therapy or from disease, in mg/L, as cited in references listed in parentheses.

$^c$Medically significant interference, defined as $\leq 2$ mg/L change for creatinine, as determined with the Seralyzer.

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on adjusting the Seralyzer mean from the comparison study for those samples with total bilirubin >5 mg/L, the new mean was 12.7 mg/L.

The Seralyzer showed a linear interference by protein, described by the regression equation \( y = 0.0406x + 0.194 \), with \( x \) = the concentration of protein (mean = 52.7 g/L), \( n = 6 \), \( r = 0.9989, S_{xy} = 0.0714 \) mg of creatinine, standard error of the slope = 9.5 \( \times 10^{-4} \), and standard error of the \( y \)-intercept = 0.058 mg of creatinine. The interference due to protein is 2.6 mg/L at a protein concentration of 60 g/L.

By analysis with the smac, the Seralyzer low calibrator had a total protein concentration of 43 g/L and total bilirubin of 5 mg/L, the high calibrator a total protein of 70 g/L and total bilirubin of 6 mg/L. (The average total bilirubin concentration for patients in our study, as determined with the smac, was 5 mg/L.)

### Analytical Variables

**Linearity.** The Seralyzer gives a linear relation for creatinine in aqueous solution, the linear regression equation being \( y = 0.986x - 2.6 \) mg/L, where \( x \) is the concentration of the standard, \( n = 10 \), \( r = 0.9995, S_{xy} = 1.097 \) mg/L, SE slope = 0.011, and SE intercept = 0.38 mg/L.

**Precision.** For serum creatinine, the run-to-run CVs were 6.1% (mean 10.8 mg/L, \( n = 24 \)) and 2.7% (mean 54 mg/L, \( n = 26 \)) for the Seralyzer; 8.9% (mean 9 mg/L) and 2.3% (mean 81 mg/L) for the smac, 5.0% (mean 8 mg/L) and 1.2% (mean 79 mg/L) for the astra, and 3.7% (mean 13.6 mg/L) for the acc.

For urinary creatinine, the run-to-run CVs were 9.9% (mean 1070 mg/L) for the Seralyzer, 6.1% (mean 1100 mg/L) for the AutoAnalyzer, 7.4% (mean 1030 mg/L) for the astra, and 6.8% (mean 1090 mg/L) for the acc \( (n = 11 \) for all methods). For creatinine clearance, the calculated CVs were 11.6% for the Seralyzer, 10.8% for the smac and AutoAnalyzer, 8.9% for the astra, and 7.7% for the acc (mean creatinine concentrations were approximately 10 mg/L for serum and 1000 mg/L for urine).

**Accuracy.** The values for the Serum 909 and the low calibrator agree well among all the methods, but not so well for the high calibrator (Table 3).

### Discussion

As mentioned, the Seralyzer method differs from the three other methods. Nevertheless, the differences between the Seralyzer results for serum and urinary creatinine as compared with the other analytical systems are probably not of medical importance. The difference for serum creatinine is a constant bias (y-intercept) of 1.0 mg/L (Seralyzer compared to the Technicon) and should not affect clinical decisions, being less than the error from random variation. Mean urinary creatinine values for the Seralyzer were closest to the mean for the acc, but were 5% lower than the mean for the AutoAnalyzer and 4% higher than that for the astra.

The results for creatinine clearance with the Seralyzer method are significantly lower than with the other three systems. Even though the serum and urinary creatinine means for the acc and astra as compared with those for the Technicon method are significantly different statistically, except for the acc serum mean, the creatinine clearance values are not, being 1.5% different for the astra and 3% different for the acc (Table 1). The difference between the means for creatinine clearance for the acc and the astra is not statistically significant (Table 1). The linear-regression analysis (Figure 1) shows that the Seralyzer mean serum creatinine results are always 1.0 mg/L higher than the AutoAnalyzer results. The ratio of the slopes of urinary to serum creatinine is 0.978 for the acc, 0.973 for the astra, and 0.963 for Seralyzer 1; urinary creatinine fails to compensate for the higher serum creatinine with the Seralyzer method. On a proportional basis, the creatinine clearance calculated from values determined with the Seralyzer would always be the lowest among the methods compared.

The difference between the Seralyzer results for creatinine clearance and the other analytical systems is of medical importance. A 12 to 15% underestimate of creatinine clearance is clinically significant, because there would be an increase in the number of patients deemed to be in renal insufficiency and for certain drugs the dosage would be decreased. For example, when the creatinine clearance falls below 100 mL/min per 1.73 m², the dosage of gentamicin, tobramycin, and amikacin must be reduced; below 80 mL/min per 1.73 m², the loading dosage of digoxin must be reduced; and below 50 mL/min per 1.73 m², the dosage of bleomycin, methotrexate, and mithramycin must be reduced 25% (7, 8). Erroneous conclusions concerning renal function could be reached if one measured creatinine clearance by the Seralyzer on one day and by a different method on another. There would be problems if one measured creatinine in one body fluid (the serum or urine) with the Seralyzer, and another body fluid by a different method. If one calculates creatinine clearance from determinations made by the Seralyzer, one should establish a reference interval for the system.

The higher mean value for serum creatinine determined with the Seralyzer in our sample as compared with the other methods appears to be related to the Seralyzer's sensitivity to bilirubin (Table 2), its accuracy being good with the Serum 909, and the calibration material not contributing to the bias (Table 3). The Seralyzer has a constant bias of 1.0 mg/L compared with the smac, but it also has an interference due to bilirubin. The calculated interference caused by bilirubin with the Seralyzer is between 0.09 and 0.15 mg of creatinine per milligram of bilirubin. At the average value for total bilirubin (5 mg/L for our patients), the maximum interference would be 0.75 mg of apparent creatinine per liter, which is less than the random error of the method, but with total bilirubin >5 mg/L a higher mean value for creatinine would emerge compared with methods that are less susceptible to bilirubin interference. In the Seralyzer, bilirubin appears to react with the reaction pad, probably the 3,5-dinitrobenzoate; bilirubin's colorimetric absorption cannot contribute, because the Seralyzer measures the rate of reaction. Less-polar solvents increase the interference caused by bilirubin, which suggests a direct reaction of bilirubin with the reagents in the pad, the non-polar solvents dissociating the bilirubin from albumin. The portion of our samples with the Seralyzer creatinine >1 mg/L than the smac creatinine and a total bilirubin >5 mg/L (the concentration in the calibrators) can account for 0.5 mg/L of the constant bias for creatinine, implying that bilirubin's reaction with the Seralyzer is the main reason for the higher

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**Table 3. Serum Creatinine Concentrations (mg/L) in Serum Reference Materials and Seralyzer Calibrators**

<table>
<thead>
<tr>
<th>Calibrators</th>
<th>Low calibrator</th>
<th>High calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SMAC</strong></td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td><strong>acc</strong></td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td><strong>ASTRA</strong></td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td><strong>Seralyzer</strong></td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup>n = 12, SD = 0.1 mg/L. <sup>b</sup>Assigned value.

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mean serum creatinine as determined with the Seralyzer in our study.

We found the level of interference caused by cephalosporin antibiotics to be essentially the same whether determined with the Seralyzer, the aca, or astra. This contradicts the suggestion by Parekh and Sims (9) that creatinine determinations with 3,5-dinitrobenzoate would show less interference with the cephalosporin antibiotics than methods involving picrate. Cefamandole, cefazolin, cephalaxin, cephaloridine, cephalothin, and moxalactam interfered more with the Seralyzer than with the astra or aca, while the interference caused by cefoxitin was 9% less than with the aca and 26% more than with the astra.

To aid in the evaluation of the medically significant interferences, we included the concentration of compounds expected from disease or treatment (Table 2). One can calculate the interference as (slope \times interferent concentration)/100. For a patient with a cefoxitin concentration of 200 mg/L, the antibiotic falsely increases the creatinine result by 9 mg/L with the Seralyzer, 7 mg/L with the astra, and 10 mg/L with the aca. Medically significant interferences with the Seralyzer are caused by cefoxitin, cefamandole, acetohexamide, bilirubin, and acetone. Thus appropriate measures should be taken to minimize the effects of these interfering compounds when one is determining creatinine.

The Seralyzer determination of creatinine in urine has a constant bias (y-intercept) of −19 mg/L, which can be explained by the positive interference of serum protein. Because serum protein reacts with the Seralyzer reaction pad, as it also does with picrate-based methods, and contributes to the slope of absorbance per unit time, the contribution of protein to the absorption slope must be subtracted from the total slope. Most manufacturers assume a constant concentration of protein in serum, determine this concentration's contribution to the absorption slope, and subtract it from the total. The value the manufacturers use usually is equal to the y-intercept of a linear regression of aqueous solutions of creatinine, which for the Seralyzer is 2.6 mg of creatinine per liter, equivalent to 60 mg of protein per liter. Since one must dilute urine 10-fold in the Seralyzer method, the y-intercept adjustment for protein becomes 2.6 mg/L. Using 12.7 mg creatinine per liter for the serum mean and adding 26 mg/L to the urine mean (assuming a volume/time of 1.31 mL/min), we calculate an adjusted creatinine clearance of 69.4 mL/min for Seralyzer 1 and 70.0 mL/min for Seralyzer 2, which are less than 6% below the Technicon value and 3% below the aca value.

In conclusion, we found determination of creatinine with the Seralyzer to have acceptable precision, good repeatability between technologists, and relative ease of performance for small batches of samples, compared to the other systems. It is suited for urgent use, requiring only several minutes per determination, once calibrated. Its only major drawback is its higher values for serum and lower values for urine, which make it unsuitable for calculating creatinine clearance. If used for serum or urine determinations alone, no appreciable medically significant differences should be noted.

We thank the Ames Co. for providing the Seralyzer reflectometer and the necessary reagents for these studies, and Mark Ruddell for the computer graphics.

References