Determination of formalin in animal and poultry inactivated vaccines using different methods

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In this study, two methods for determination of formalin amount were applied on samples of inactivated vaccines representing local or foreign companies. The first method; matching method was conducted by using phenyl hydrazine while in the other method is; spectrophotometry phloroglucinol was used. Spectrophotometrical method was found more sensitive and more accurate than the matching one. At the same time, the spectrophotometry method could be used for determination of formalin in all inactivated vaccines either bacterial or viral vaccines.

During vaccine production, several control tests are performed to ensure that vaccines have been made under optimal circumstances; the raw and intermediate products follow the required standard. At the end of the production process, many tests must be done on the bulk product and the final vaccine. Such tests include safety, potency, sterility, identity as well as residual chemical constituents particularly formalin which is used as inactivating agent during manufacture. Formalin is considered as toxigenic or carcinogenic material for both animals and human beings. Tests have been formulated for each vaccine according to (British Pharmacopoeia, 1973; Quality Control of Vaccine, 1983 and European Pharmacopoeia, 1997) which are generally accepted allover the world.

Brandly et al. (1946) showed that 0.025% diluted formalin could destroy the infectivity of Newcastle disease virus (NDV).

King (1991) evaluated different methods for inactivation of NDV and avian influenza viruses (AIV). He found that NDV or AIV were inactivated by binary ethyleneimine (BEI) (0.01M) with no adverse effect on haemagglutinating or hemolytic activities. The effect of formalin (0.1%) was variable and depressed HI titres of antisera.

Soliman et al. (1996) found that binary ethylenimine, used as inactivating agent for NDV, had no effect on the antigenicity of the virus when used in a concentration of 0.01 or 0.03 M. These results compared favourably with those obtained when using formaldehyde solution as an inactivating agent at concentration of 0.1 or 0.2%, which significantly reduced antigenicity of the haemagglutination titer of 1024 to that of 32.

The widespread use of formaldehyde and the reports of adverse effects have created the need for specific, sensitive and simple method for its determination.

The present work aimed to compare between two currently used methods for determination of formalin; the first one is by using phenyl hydrazine and the other is by using phloroglucinol (spectrophotometry).

Material and Methods

Tested batches. Total number of (136) batches of inactivated vaccines were used in this study. These batches represent local and imported vaccines; (88) viral poultry inactivated vaccines, (29) bacterial poultry inactivated vaccines, (10) viral large animal inactivated viral vaccines and (9) inactivated bacterial vaccines of large animal.

Phenyl hydrazine method. It was carried out according to Quality Control of Vaccines (1983). Standard formaldehyde solution was prepared by measuring out 1 ml of aqueous formaldehyde solution (40%) into a 100 ml standard flask and diluting to the mark with distilled water. The concentration of the solution was found to be 3800 ppm. One ppm of formaldehyde solution was prepared freshly prior to use. In five test tubes represented five concentrations (1.0, 0.50, 0.1, 0.05 % and 0.01 %) of the formaldehyde (El-Gomhoria Company for Chemicals, Drugs and Medical Supplies, Egypt) standards. 1ml in each tube and another test tube represented the sample (1ml of the tested inactivated vaccine plus 100 ml distilled water), the following reagents were put in order: 0.1 ml of phenyl hydrazine 1% (Riedel-de Haen Allied Signal, Germany), 0.1 ml of potassium ferricyanide 5%(BDH Chemicals Ltd., Poole, England) and Three drops of concentrated hydrochloric
acid (HCl) (The Egyptian Company for
Chemicals and Drugs (ADWIA), Egypt). After 5
min. the colour of both tubes was matched.

2. Phloroglucinol method. It was performed
according to the method described by (Gayathri
and Balasubramanian, 2000):

Preparation of Calibration Graph. In five test
tubes containing 1 ml of 1% phloroglucinal
(BDH Chemicals Ltd., Poole, England), 0.5, 1.0,
1.5, 2.0 and 2.5 ml of 1 ppm standard formald-</ns>
hyde solution was added separately. 4 ml
centrated sulphuric acid (ADWIA Company,
Egypt.) was added carefully to each tube using a
long stem funnel. The solution was allowed to
stand for 20 min. to attain room temperature.
The solution was then transferred into 10 ml
standard flask, washed with 1ml of 9 M
sulphuric acid and diluted to the mark with the
same acid. Absorbance was measured at 435 nm
against reagent blank prepared according to the
same procedure (Table 1, Fig. 1).

Determination of formaldehyde in inactivated
vaccines. One ml of the inactivated vaccine was
diluted to 100 ml with distilled water. The
diluted solution was filtered and used for
analysis. One ml of the sample solution was
taken and analyzed for formaldehyde content
following the procedure described under

Calculation. Calculation of formaldehyde
concentration (ppm) was obtained from the
calibration graph based on the following
equation:

\[ \frac{\Delta \text{OD (Sample)}}{\Delta \text{OD (Standard)}} = \frac{X}{\text{Concentration of standard}} \]

Results and Discussion

Several methods for determination of
formaldehyde have been developed, e.g. high
performance liquid chromatography (HPLC)
method (Zegota, 1999; Sandner et al., 2001 and
Possanzini and Di Pola, 2003), gas
chromatography (GC) method (Ren and Guo,
1997; Suliman and Soma, 2000 and Shiraishi
et al., 2001), laser spectrometer (Rehle et al., 2001
and Richter et al., 2002) and by
spectrophotometry (Lodge, 1989; Lancaster et al.,
2000; Ross et al., 2002 and Mason et al., 2004).

In this study, two methods were used; the
principle of the first method is base on the reaction
of formaldehyde with phenyl hydrazine solution
(1%), potassium ferricyanide (5%) in acid solution
(HCl) forming a red to faint pink coloured
compound, the intensity of which can be matched
visually with 1, 0.50, 0.1, 0.05 and 0.01% of
formaldehyde standard solutions (Quality Control
of Vaccines, 1983). The second method is based
on the reaction of formaldehyde with
phloroglucinol in acidic solution (Gayathri and
Balasubramanian, 2000).
Table (2): Determination of formaldehyde concentration (%) in random batches of inactivated vaccines using visual method (Matching).

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of batches</th>
<th>Mean±SE</th>
<th>Type</th>
<th>No. of batches</th>
<th>Mean±SE</th>
<th>Type</th>
<th>No. of batches</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
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<td>0.044±0.002</td>
<td>Cholera</td>
<td>9</td>
<td>0.049±0.007</td>
<td>RVF</td>
<td>3</td>
<td>0.05±0.0</td>
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<tr>
<td>IBD</td>
<td>8</td>
<td>0.05±0.0</td>
<td>Coryza</td>
<td>10</td>
<td>0.03±0.006</td>
<td>AHS</td>
<td>2</td>
<td>0.05±0.0</td>
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<td>EDS</td>
<td>5</td>
<td>0.038±0.007</td>
<td>FRHS</td>
<td>6</td>
<td>1.0±0.0</td>
<td>Entero-3</td>
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<td>0.01±0.0</td>
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<td>RVHS</td>
<td>4</td>
<td>0.05±0.0</td>
<td>MG</td>
<td>2</td>
<td>0.01±0.0</td>
<td>FMD</td>
<td>3</td>
<td>0.01±0.0</td>
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<tr>
<td>ND+TRT</td>
<td>2</td>
<td>0.53±0.48</td>
<td>E. coli</td>
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<td>ND+IB</td>
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<tr>
<td>ND+IBD</td>
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<tr>
<td>ND+EDS</td>
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<td>0.1±0.0</td>
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<tr>
<td>ND+IB+EDS</td>
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<td>0.05±0.0</td>
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<tr>
<td>ND+IB+SHS</td>
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<td>ND+IB+IBD</td>
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<td>0.05±0.0</td>
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<td></td>
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<tr>
<td>ND+IB+IBD+Reo</td>
<td>3</td>
<td>0.04±0.01</td>
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<tr>
<td>PPMV</td>
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<td>0.01±0.04</td>
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</tr>
<tr>
<td>AE</td>
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<tr>
<td>Total</td>
<td>88</td>
<td>0.12±0.03</td>
<td>29</td>
<td>0.24±0.07</td>
<td>10</td>
<td>0.039±0.007</td>
<td>9</td>
<td>0.057±0.009</td>
</tr>
</tbody>
</table>

Mean ± Standard Error (SE)
Table (3): Determination of formaldehyde concentration (%) in random batches of inactivated vaccines using spectrophotometry method.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>No. of batches</th>
<th>Mean±SE</th>
<th>Type of vaccine</th>
<th>No. of batches</th>
<th>Mean±SE</th>
<th>Type of vaccine</th>
<th>No. of batches</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
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<td>Viral Vaccines</td>
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</tr>
<tr>
<td>ND</td>
<td>10</td>
<td>0.0465±0.001</td>
<td>Cholera</td>
<td>5</td>
<td>0.0522±0.001</td>
<td>RVF</td>
<td>3</td>
<td>0.0518±0.003</td>
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<tr>
<td>IBD</td>
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<td>0.0521±0.002</td>
<td>Coryza</td>
<td>5</td>
<td>0.0352±0.001</td>
<td>AHS</td>
<td>2</td>
<td>0.0507±0.003</td>
</tr>
<tr>
<td>EDS</td>
<td>3</td>
<td>0.0395±0.001</td>
<td>FRHS</td>
<td>3</td>
<td>1.0209±0.02</td>
<td>Entero-3</td>
<td>2</td>
<td>0.0171±0.002</td>
</tr>
<tr>
<td>RVHS</td>
<td>3</td>
<td>0.0532±0.001</td>
<td>MG</td>
<td>2</td>
<td>0.0159±0.0002</td>
<td>FMD</td>
<td>3</td>
<td>0.0016±0.0003</td>
</tr>
<tr>
<td>ND+TRT</td>
<td>2</td>
<td>0.0534±0.003</td>
<td>E. coli</td>
<td>2</td>
<td>0.0151±0.0003</td>
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</tr>
<tr>
<td>ND+IB</td>
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<td>0.0563±0.002</td>
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<tr>
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<td>ND+IB+EDS</td>
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<td>0.0507±0.001</td>
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<tr>
<td>ND+IB+SHS</td>
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<td>0.0269±0.002</td>
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<tr>
<td>ND+IB+IBD</td>
<td>3</td>
<td>0.0516±0.001</td>
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</tr>
<tr>
<td>ND+IB+IBD+Reo</td>
<td>3</td>
<td>0.0446±0.001</td>
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<tr>
<td>PPMV</td>
<td>2</td>
<td>0.0155±0.001</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AE</td>
<td>2</td>
<td>0.0301±0.0002</td>
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<tr>
<td>Total</td>
<td>45</td>
<td>0.0462±0.002</td>
<td></td>
<td>17</td>
<td>0.2095±0.09</td>
<td></td>
<td>10</td>
<td>0.0296±0.007</td>
</tr>
</tbody>
</table>

Mean ± Standard Error (SE)

Table (4): Comparison between determination of formaldehyde concentration (%) in random batches of inactivated vaccines using visual method (Matching) and spectrophotometry method.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Visual method (matching)</th>
<th>Spectrophotometry method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of batches</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Viral poultry inactivated vaccines</td>
<td>88</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Bacterial poultry inactivated vaccines</td>
<td>29</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>Viral large animal inactivated vaccines</td>
<td>10</td>
<td>0.039 ± 0.007</td>
</tr>
<tr>
<td>Bacterial large animal inactivated vaccines</td>
<td>9</td>
<td>0.057 ± 0.009</td>
</tr>
</tbody>
</table>

Mean ± Standard Error (SE)
The obtained results were illustrated in (Tables 2-4) which showed determination of formaldehyde percent in 136 random batches of inactivated vaccines used in this study, which were collected during the period of (2004-2005). These batches represent local and imported vaccines including 88 viral poultry inactivated vaccines, 29 bacterial poultry inactivated vaccines, 10 viral large animal inactivated vaccines and 9 bacterial large animal inactivated vaccines.

From the obtained results, it was noted that the mean percentage of formaldehyde of the viral poultry inactivated vaccines was (0.0462 %) by spectrophotometrical method and (0.12 %) by the visual method. On the other hand, formaldehyde mean percentages in bacterial poultry inactivated vaccines were 0.2095 and 0.24% in the spectrophotometrical and in visual methods respectively. The viral large animal inactivated vaccines show mean percentage of 0.0296 % and 0.039 % by spectrophotometrical and visual methods respectively. Bacterial large animal inactivated vaccines showed formaldehyde mean percentage of 0.0579 and 0.057 % by spectrophotometrical and visual methods respectively.

Although the two methods gave nearly the same values, it is very clear that the spectrophotometry method is more sensitive than the visual one, where the former gave very accurate percentage of formaldehyde while the latter gave approximate values. The obtained results are in agreement with those obtained by (Gayathri and Balasubramanian, 2000) who said that the determination of formaldehyde spectrophotometrically using phloroglucinal is a simple, accurate and very sensitive method, also with Ross et al. (2002) who conducted an international collaborative study of quantitative colorimetric method for determination of formaldehyde in veterinary vaccines products by 15 laboratories in North America, Europe and Japan. Moreover, Amer (2004) showed that the developed spectrophotometry method using Rosaniline was conveniently applied to the determination of traces of formaldehyde in veterinary biological products.

In conclusion, the determination of formaldehyde spectrophotometrically using phloroglucinol method is more simple, accurate and sensitive than the visual (matching) method. The spectrophotometry method could be used for determination of formaldehyde not only in the poultry viral inactivated vaccines but also in all veterinary inactivated vaccines either bacterial or viral.

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References


