A cytological and histological study of acute premyelocytic leukaemia

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SYNOPSIS Among the acute leukaemias of the granulocytic group, acute premyelocytic leukaemia is distinguished by the severity of its haemorrhages, the frequency of hypofibrinaemia, a rapidly fatal course, and an unusual cellular hyperplasia. Myelograms show an increased proportion (average 80%) of characteristic cells of large diameter, with numerous azurophil granules. The infiltration of other organs is inconstant.

MATERIAL AND METHODS

Acute premyelocytic leukaemia represents about 6.8% of all cases of acute leukaemia seen at our Institute (Bernard, Boiron, Weil, Levy, Seligmann, and Najean 1962). Between 1956 and August 1962, 34 cases of acute premyelocytic leukaemia from a total of 497 cases of acute leukaemia were seen. We have chosen for this study 25 cases followed up until death with, in 12 cases, a full necropsy.

The diagnostic criterion used was the presence of a very large proportion of cells of the premyelocyte type (over 50% and often 80 to 100%) in the bone marrow, associated with the clinical and haematological features of acute leukaemia.

Cytological studies were made on bone marrow and blood smears stained by the May-Grünwald-Giemsa method. The histological features of the bone marrow and various viscera obtained at necropsy were examined in tissues fixed in Bouin's fluid and stained with haematoxylin-phloxin-saffron.

HAEMATOLOGICAL STUDY

The clinical characteristics of acute premyelocytic leukaemia were first described in a previous communication (Bernard, Mathé, Boulay, Céoara, and Chomé, 1959). Table I presents the principal haematological data on the present series of cases. Anaemia was generally intense with a red cell count at first examination of under 2.5 million per c.mm. in 18 cases. The leucocyte count was variable, but often diminished (less than 5,000 c.mm. in 17 cases). Premyelocytes were found in the blood in 21 of the cases, but their number showed considerable individual variations. The myelogram as a rule showed a massive increase in the premyelocytes, which made up an average of 80% of the medullary elements; in three cases, however, their concentration was between 50% and 70% (cases 2, 8, 19).

Thrombocytopenia was found in 23 out of 24 cases and was usually considerable (less than 50,000 platelets per c.mm. in 18 of the cases). Rapid fibrinolysis was seen in 10 out of 20 cases in which it was studied; considerable hypofibrinaemia was seen in 10 cases out of 11.

The course was as a rule acute, and remissions were exceptional. In 21 of the 24 cases in which the survival time could be evaluated, it was shorter than a month. Death was generally associated with haemorrhagic manifestations.

CYTOLOGICAL STUDY

The malignant premyelocyte is a cell of considerable size (15 to 20 μ), characterized even when only slightly magnified by a great number of granulations, which sometimes give it the appearance of a muri-form cell. When examined at a high magnification, it is clearly distinct from the myeloblast. The nucleus is large and often eccentric. Its chromatin is less dense than that of the normal premyelocyte, and distinguished from that of the myeloblast by its coarser texture and greater density. Some of these cells are nucleolated but the majority are not. It is difficult, however, to make an exact study of all the nuclei, because some are almost completely covered by the cytoplasmic granulations. The cytoplasm is abundant and basophile or, less frequently, lilac coloured. The azurophil granules cover almost the entire surface of the cell; some specific granulations

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can be observed among them. Anomalous by their number alone, the cytoplasmic granulations are usually abnormal also by their size, which exceeds that of the granules in normal premyelocytes. A few cells may even contain enormous polyhedral azurophil granulations. In one case (no. 24) most of the cells contained accumulations of Auer bodies.

Other malignant elements seen include haemocytoblasts, myeloblasts, and particularly monocytoid haemoblasts, the last always in small numbers.

**HISTOLOGICAL STUDY**

Macroscopically haemorrhagic lesions, visceral or cerebromeningeal, were not seen more frequently than in other varieties of leukaemia, contrary to what might have been expected on the basis of the clinical observations. The principal results obtained by histological examination are presented in Table II.

**BONE MARROW** The features of the marrow sections are highly characteristic because of the massive and uniform infiltration with fairly large (15 to 20 μ), and very unusual cells which have clearly delineated round or oval contours, with a rounded, eccentric nucleus and very dense chromatin. A few mitoses are noted. The cytoplasm, of a rusty colour reminiscent of haematoxylin-phloxin-saffron staining, is granular but contains no distinguishable inclusions or special staining affinity. By their diameter, chromat in, and cytoplasmic colour, these cells are distinct from the plasmocytes which at first sight they resemble but which lack these characteristic features. There is almost absolute uniformity, although undifferentiated leucoblastic elements are sometimes observed. Four cases showed sparse islets of erythroblasts (cases 1, 13, 14, and 24). The leukaemic proliferation provokes no invasion of bone, and the cortical layers and spicules are normal. Only one of our cases (no. 15) did not fulfil this criterion because death took place during a complete remission.

**FREQUENCY OF INVOLVEMENT OF OTHER ORGANS** This frequency was estimated on the basis of 11 necropsies. Infiltration of organs other than the bone marrow was found to be inconstant. It was impossible to calculate the frequency exactly, because not all the necropsies were complete, although the spleen and liver (11 cases), kidneys and lungs (9 cases), and

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### TABLE I

**HAEMATOLOGICAL DATA IN PRESENT SERIES**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Haemogram</th>
<th>% of Precyto-</th>
<th>Haemostasis</th>
<th>Remission</th>
<th>Duration</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>White Cell Count (per c.mm.)</td>
<td>Red Cell Count (per c.mm.)</td>
<td>% of Pre-</td>
<td>Platelets (per c.mm.)</td>
<td>Fibrinolysis</td>
<td>Hypofibrinemia</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>F</td>
<td>18,000</td>
<td>1-9</td>
<td>42</td>
<td>71</td>
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<td>+</td>
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<tr>
<td>2</td>
<td>8</td>
<td>F</td>
<td>6,400</td>
<td>1-3</td>
<td>44</td>
<td>60</td>
<td>35,000</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>F</td>
<td>4,600</td>
<td>2-3</td>
<td>40</td>
<td>100</td>
<td>35,000</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>F</td>
<td>2,000</td>
<td>1-4</td>
<td>22</td>
<td>80</td>
<td>40,000</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>F</td>
<td>234,000</td>
<td>1-6</td>
<td>98</td>
<td>93</td>
<td>45,000</td>
<td>+</td>
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<tr>
<td>6</td>
<td>32</td>
<td>F</td>
<td>8,200</td>
<td>1-4</td>
<td>76</td>
<td>100</td>
<td>25,000</td>
<td>/</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>M</td>
<td>3,400</td>
<td>1-9</td>
<td>62</td>
<td>70</td>
<td>35,000</td>
<td>+</td>
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<tr>
<td>8</td>
<td>53</td>
<td>F</td>
<td>17,600</td>
<td>3-2</td>
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<td>50</td>
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<td>9</td>
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<tr>
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<td>/</td>
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<td>12</td>
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<td>7</td>
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<td>F</td>
<td>1,400</td>
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<td>93</td>
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<td>18</td>
<td>18</td>
<td>M</td>
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<td>40,000</td>
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<tr>
<td>19</td>
<td>47</td>
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<td>55</td>
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<td>21</td>
<td>M</td>
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<td>30</td>
<td>100</td>
<td>30,000</td>
<td>0</td>
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<tr>
<td>21</td>
<td>13</td>
<td>M</td>
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<td>96</td>
<td>160,000</td>
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<td>14</td>
<td>F</td>
<td>2,800</td>
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<td>78</td>
<td>60,000</td>
<td>+</td>
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<tr>
<td>23</td>
<td>5</td>
<td>M</td>
<td>3,800</td>
<td>2-8</td>
<td>37</td>
<td>70</td>
<td>100,000</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>17</td>
<td>F</td>
<td>1,800</td>
<td>3-1</td>
<td>0</td>
<td>96</td>
<td>85,000</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>45</td>
<td>M</td>
<td>8,600</td>
<td>2-5</td>
<td>77</td>
<td>90</td>
<td>45,000</td>
<td>+</td>
</tr>
</tbody>
</table>

/ = no specimen

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### Table II

**HISTOLOGY OF PREMYELOCYTIC LEUKAEMIA IN 11 CASES**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Bone Marrow</th>
<th>Spleen</th>
<th>Lymph Node</th>
<th>Liver</th>
<th>Kidney</th>
<th>Perirenal Tissue</th>
<th>Adrenals</th>
<th>Lungs</th>
<th>Brain</th>
<th>Peripheral Nerve</th>
<th>Ovary or Testicle</th>
<th>Uterus and Tubes</th>
<th>Heart</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ Massive premyelocytes</td>
<td>+ Red pulp polymorphs</td>
<td>+ Sinus polymorphs</td>
<td>+ Periportal sinusoides capillaries polymorphs</td>
<td>+ Premyelocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+ Massive premyelocytes</td>
<td>0</td>
<td>0</td>
<td>+ Periportal polymorphs</td>
<td>+ Massive haemorrhage foci polym.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+ Massive premyelocytes</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Alveolar walls, vessel undifferentiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+ Massive premyelocytes</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Alveolar walls, vessel undifferentiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+ Massive premyelocytes</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Massive, undifferentiated</td>
<td>+ Alveolar walls, vessel undifferentiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+ Massive premyelocytes</td>
<td>+ Red pulp undifferentiated</td>
<td>+ Periportal undifferentiated</td>
<td>+ /</td>
<td>0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+ Massive premyelocytes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>+ Massive premyelocytes</td>
<td>+ Red pulp polym.</td>
<td>+ Portal vessels (parenchyma)</td>
<td>+ Perivascular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+ Massive premyelocytes</td>
<td>+ Red pulp premyelocytes</td>
<td>+ Red pulp polym.</td>
<td>+ Massive premyelocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes:

- + = leukaemic infiltration
- 0 = no infiltration
- / = no specimen
- Massive = complete, total infiltration
- Partial = leukaemic infiltrates localized in some areas (periportal, liver), (red pulp of spleen)
- Premyelocytes = leukaemic cells which are exclusively or largely premyelocytes
- Undifferentiated = leukaemic cells not cytologically identified
- Polym. = besides undifferentiated leukaemic cells, presence of identifiable premyelocytes and often more mature myeloid cells
FIG. 1. Premyelocytic infiltration of the bone marrow. Haematoxylin-phloxin-saffron × 820.

FIG. 2. Premyelocytes as they appear under high-power magnification in bone marrow sections. Haematoxylin-phloxin-saffron × 1,500.


FIG. 4. Premyelocytic infiltrate in the perirenal fat tissue. Haematoxylin-phloxine-saffron × 150.
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FIG. 5. Premyelocytic infiltrate in the perirenal fat tissue (same as Fig. 4). Haematoxylin-phloxine-saffron × 820.


FIG. 7. Leukaemic premyelocytes (bone marrow smear). May-Grünwald-Giemsa × 1,500.

lymph nodes (7 cases) were examined sufficiently systematically to warrant conclusions. The organs most frequently affected were the spleen (9 cases out of 11) and the liver (8 cases out of 11). The lungs (3 out of 9), lymph nodes (3 out of 7), kidneys (1 out of 9), and perirenal zone (3 out of 9) were less frequently affected. A leukaemic meningeal focus was found in two cases; leukaemic infiltrations in a cerebral haemorrhagic focus in the uterine muscle (case 5), in the heart, around a peripheral nerve, and in perihypophyseal vessels were also found. In one case (no. 2) there was a massive infiltration of the striated muscles near a haemorrhagic focus.

TYPE OF INFILTRATION Unlike those of the bone marrow, the leukaemic infiltrates of the viscera are not uniform. Only in three specimens (case 1, perirenal zone; case 2, liver and spleen) were premyelocytes found almost exclusively. In all other cases the visceral infiltrates were pleomorphic; premyelocytes were identifiable, often in large numbers, but always in the company of other cells which could not be cytologically classified, as is the rule in histological sections. These cells could only be recognized as undifferentiated haemoblasts. Their features were variable; they showed an irregular, large nucleus, less dense than that of the premyelocytes, with relatively less abundant cytoplasm. Occasionally, more mature myeloid cells were also seen (metamyelocytes, granulocytes). In some cases no premyelocytes could be identified in the visceral infiltrates; the features in these cases were completely undifferentiated.

TOPOGRAPHY OF INFILTRATION In one case (no. 5), there was massive visceral infiltration of the principal organs (liver, spleen, kidneys, perirenal zone,
lymph nodes, lungs, uterus) but in all other cases visceral infiltration was incomplete.

Spleen  The splenic structure remained recognizable but the white pulp was hypoplastic; the infiltration was in the red pulp. In only one case was total infiltration seen (no. 25).

Liver  Infiltrates were periportal and often very small; the leukaemic cells sometimes infiltrated the sinusoid capillaries.

Perirenal tissue  In three cases infiltration was found, sometimes near a haemorrhagic focus.

Lungs  Infiltration was localized in the alveolar walls; the vessels were stuffed with leukaemic cells.

Lymph nodes  In the various lymph nodes obtained after death infiltration was either massive, with capsular and pericapsular invasion, or limited, more or less effacing the lymph node structure but not going beyond the capsule.

Other visera  The cerebrum and cerebellum may be infiltrated. The leucocytic cells infiltrated the haemorrhagic foci and the meninges and filled the vessels. In the uterus, infiltration was localized among the muscle fibres. The vessels of the ovaries were stuffed with leucocytic elements.

NON-SPECIFIC LESIONS  Non-specific lesions were frequently found. In the spleen, haemorrhagic foci, congestion, and the stigmata of haemolysis are nearly always found. In the liver vascular congestion and signs of cholestasis were sometimes seen; hepatic steatosis existed in one case (no. 24). In one case (no. 11) there was hepatic and splenic mycosis. In the lungs there may be foci of congestion, infarction, or even necrosis. Pancreatic and adrenal necrosis and congestion are also possible.

DISCUSSION

Although the diagnosis of acute premyelocytic leukaemia generally offers no difficulty, a number of problems must be considered.

Cytologically, the leukaemic myeloblast shows a younger nucleus and fine chromatin structure, nucleolated or even with several nucleoli; the cytoplasm is abundant and basophilic, containing only a few azurophil granulations and sometimes even a few Auer bodies. A few premyelocytes are occasionally found in myeloblastic leukaemia (not more than 5 to 10%). Histologically, the features differ considerably from those of premyelocytic leukaemia. The cells have a larger nucleus, with less dense chromatin and more irregular contours (notched); the cytoplasm is less abundant, with less distinct contours and variable staining characteristics; there is as a rule a tendency to basophilia but always the staining affinity differs from that of premyelocytes.

A much more difficult problem is posed by some acute leukaemias of the granulocyte group, which have the clinical features of acute premyelocytic leukaemia. Their cytological features are polymorphous in the bone marrow and the blood, in which are found haemocytoblasts, myeloblasts, monocyteid haemoblasts, premyelocytes, and even some myelocytes. In some rare cases, the percentage of premyelocytes among the medullary elements may be as high as 40. In two cases (personal observations) the premyelocytes made up 40% and 43% of the myelogram; their features were identical with those seen in typical premyelocytic leukaemia, and this diagnosis could not be eliminated by cytological examination. On the other hand, the histology of the marrow was polymorphous and did not include a premyelocytic sheet of homogeneous type, as in our other observations. We believe that there must be described as borderline cases of acute premyelocytic leukaemia.

The acute premyelocytic transformation of a chronic myeloid leukaemia is an infrequent form of acute transformation (Bernard et al., 1959). In these cases the smear contains a large number of premyelocytes, but these differ from those of acute leukaemia, showing a closer similarity to normal premyelocytes, with finer and much less numerous granulations, always permitting examination of the nucleus. The histological data do not allow differentiation of the two types of cell, but in the case of transformation of chronic myeloid leukaemia, the features are more polymorphous. The cytology and the context of a case make differentiation easy.

REFERENCES


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We wish to thank Sister Stewart, Dr. Lowry, and Dr. Neely for their cooperation and help in the collection of specimens and examination of case histories, and Dr. S. Nelson for the animal inoculations.

REFERENCES

Ling, N. R. (1960). Ibid., 77, 12P.

ADDENDUM

Since this paper was submitted for publication it is learned that Organon Laboratories have now reduced the sensitivity of their pregnancy test. A further series of tests have been carried out using this modified Pregnosticon. One false positive result was obtained from 115 non-pregnancy urines, and one false negative result from 78 pregnancy urines. These results indicate that Pregnosticon is now a satisfactory test for pregnancy diagnosis.

CORRECTION

The title of the paper by Jean Bernard, J. Lasneret, J. Chome, J. P. Levy, and M. Boiron (J. clin. Path., 16, 319) should read: 'A cytological and histological study of acute promyelocytic leukaemia'. Throughout, the nomenclature 'promyelocyte' should be used instead of 'premyelocyte'.

Standardization of haemoglobin solutions by iron determination

E. C. MASON and A. ADARRAGA-ELIZARAN From the Red Cross Blood Transfusion Service, Brisbane, Australia

Earlier evaluations of haemoglobin standards in this laboratory utilized a modification of the direct titanium sulphate microtitration of residual Fe in wet- or dry-ashed haemoglobin (McFarlane, 1932; Ramsay, 1944; O'Hagan 1957). In the spectrophotometric determination of a coloured complex formed by interaction of haemoglobin Fe with a sensitive reagent Fe is split from haemoglobin by wet-way oxidative procedures terminating in either partial destruction of the protein, requiring a subsequent filtration step (Wong, 1928; Sunderman, MacFate, MacFadyen, Stevenson, and Copeland, 1953; Dickenman, Crafts, and Zak, 1954), or in complete destruction of the protein followed by evaporation of Copeland volatile acids (Williams and Zak, 1957). Preliminary trials of the first alternative were not completely satisfactory in our hands, possibly because of partial irreversible absorption of Fe by the precipitated protein. On investigation of the second, certain modifications were introduced, including the elimination of the final evaporative step.

MATERIALS AND METHODS

REAGENTS All are made up in iron-free distilled water.

1 buffered 2,2'-dipyridyl solution One gram of 2,2'-dipyridyl is dissolved with warming in about 700 ml. water, and to this is added 300 g. anhydrous sodium acetate (A.R.). When solution is complete, the whole is transferred to a one-litre volumetric flask and made up to the mark. This solution is stored in a dark glass bottle.

2 mineral acids The concentrated acids used, H2SO4 (S.G. 1.83), HNO3, (S.G. 1.42) and HClO4 (72%), are each of A.R. quality.

3 ascorbic acid One gram is dissolved in 100 ml. water for each standardization. The solution is stored in a refrigerator when not in use but is discarded after 24 hours.

4 standard iron stock solution This contains 10 mg. Fe per ml.; 86.6 g. (Fe2(SO4)3·(NH4)2SO4·24H2O (A.R.) is dissolved in 400 ml. water, 200 ml. 10% H2SO4 is added, and the solution is transferred to a one-litre volumetric flask and diluted to the mark. The iron content is accurately established by a standard oximetric procedure (Vogel, 1951).

5 standard iron working solution Accurate 1/100 dilution of stock solution = 100 μg./ml.

APPARATUS All glassware must be free of iron. This is accomplished by cleaning with chromic acid, rinsing

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