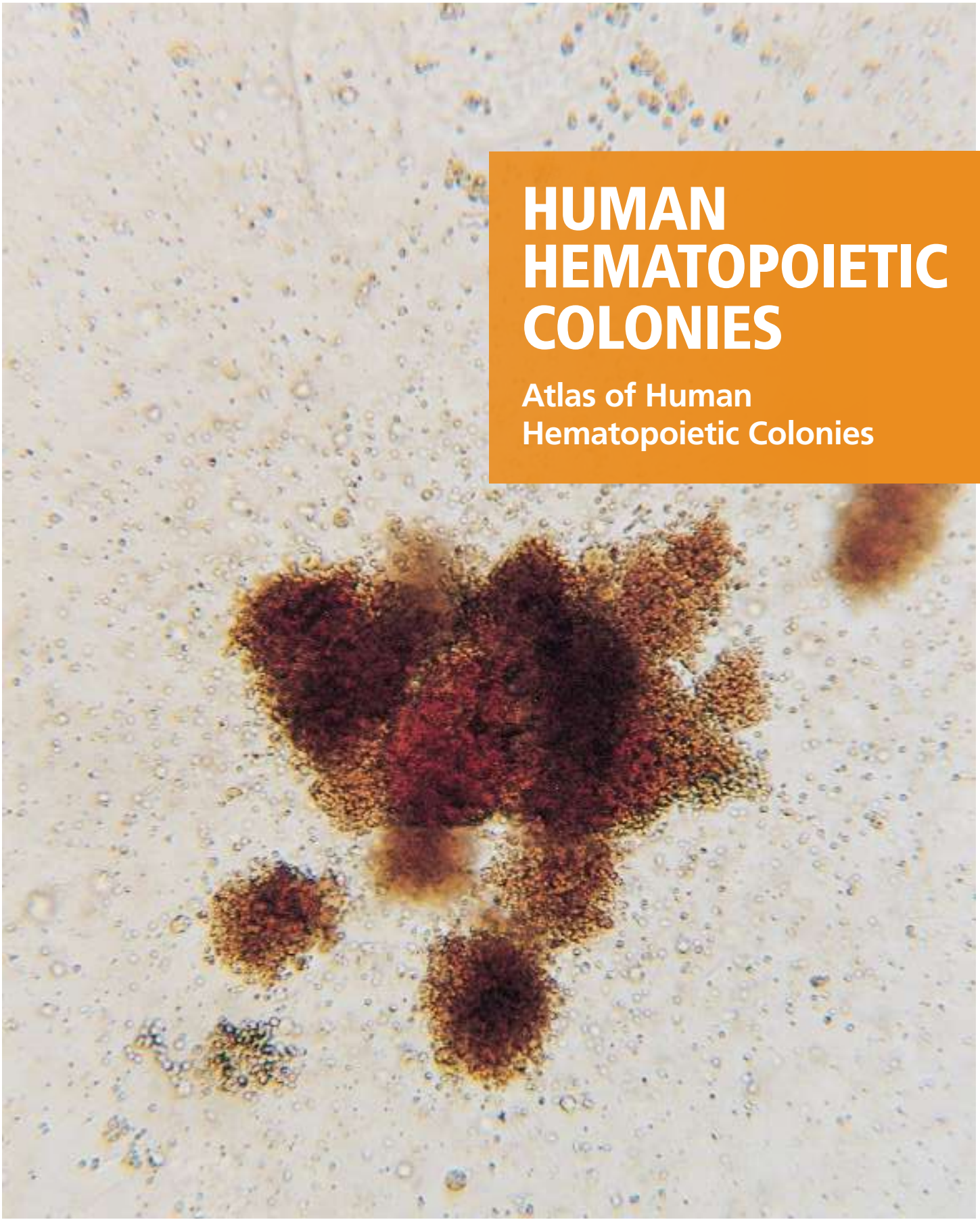

HUMAN HEMATOPOIETIC COLONIES

Atlas of Human Hematopoietic Colonies

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Atlas of Human Hematopoietic Colonies

An introduction to the recognition of colonies produced by human hematopoietic progenitor cells cultured in methylcellulose media.

- by Connie Eaves and Karen Lambie

Introduction

Mature blood cells are highly specialized and most have a life span considerably shorter than that of the human body. After birth, the production of new blood cells normally occurs primarily in the bone marrow at a rate of many billion cells per day. Although many different lineages of blood cells are now recognized, these are believed to derive throughout adult life from a common pool of totipotent stem cells. Blood cell production from these primitive hematopoietic cells is an extremely complex process involving the continuous and coordinated differentiation and proliferation of intermediate progenitor cell types under the control of a large number of regulatory cytokines, many of which are locally produced by bone marrow stromal cells. Much progress has been made during the last three decades in defining the hierarchical relationships of these progenitor cells and most of these can now be detected by in vitro colony assays.

The first colony assay for a human hematopoietic progenitor cell was described in 1967 by a group of investigators in Toronto. This was a natural extension of their pioneering studies with mice in the early 1960's which introduced the concept of identifying primitive hematopoietic cells retrospectively by stimulating them to express their developmental potential. Culture conditions have now been identified that allow the formation in vitro of colonies of erythroid cells, granulocytes, macrophages, and megakaryocytes as well as combinations of these, from single human hematopoietic cells. Detection of the clonal progeny of individual precursors present in the original suspension is made possible by plating the cells in a medium that contains a gelling agent that greatly decreases cell movement. The more mature cells that make up the majority of the cells in either blood or marrow usually lyse within a few days after being placed in culture. New cells that are produced as localized colonies are thus usually easy to identify using an inverted microscope.

The availability of reproducible and quantitative in vitro assays for a variety of different types of hematopoietic progenitor cells has been a powerful impetus to the investigation of both normal and abnormal hematopoiesis. These methods are also useful for assessing the progenitor content of marrow or blood cell populations before and after a variety of in vitro manipulations, particularly those used routinely as part of allogeneic or autologous transplant procedures.

One of the greatest problems inherent in the use of in vitro hematopoietic colony assays is the variability in both colony growth and appearance that can be encountered with slight changes in the particular batch or concentration of each of the components of the medium used. Rigorous control of each of these to provide maximal plating efficiencies and reproducible colony morphology is a key feature of the culture reagents made available through STEMCELL Technologies Inc. This allows novice and experienced investigators alike to take immediate and continuing advantage of a long standing experience in optimizing the selection and preparation of these complex media. Even with standardized culture reagents, significant variation in colony morphology occurs with different human samples, including those from different normal individuals. Moreover, additional diversity is frequently encountered when samples include progenitor cells altered by a disease process, e.g. as in patients with acute leukemia. This atlas has been prepared to serve as an introduction and guide to colony recognition and to illustrate some of the heterogeneity in colony morphology that is commonly encountered.

It is important to remember that standardization of the culture medium is only one of the conditions necessary to maximize reproducibility of hematopoietic colony formation. Other conditions, including: temperature, humidity, O2 and CO2 levels, can affect colony growth and morphology, as does the incubation time allowed before the colonies are scored. In addition, the method used to prepare the cells, their purity and concentration as well as the types of growth factors used in the assay can influence the results obtained. A description of the differences in the appearance of colonies that form in methylcellulose media containing a cocktail of recombinant growth factors (recombinant cocktail or RC) by comparison to factors present in agar-stimulated leukocyte conditioned media (typically used until recently and referred to here as Agar LCM) is included in the illustrative material contained in this atlas.

Colony Culture Media and Scoring Procedures

This atlas deals exclusively with colonies produced from hematopoietic progenitor cells present in human blood and bone marrow when these cells are cultured in appropriate methylcellulose-based media. These progenitor cells are called "colony-forming units" (CFU) or "burst-forming units" (BFU) in the case of certain erythroid progenitors and are then further classified according to the type (lineage) and number of mature cells produced in each colony as follows:

CFU-E (colony-forming unit - erythroid). Produces 8-200 erythroblasts in 1-2 clusters

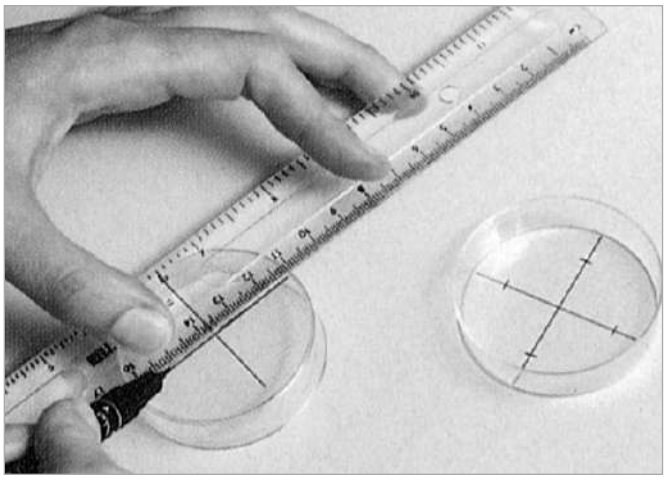
BFU-E (burst-forming unit - erythroid). Produces 3 or more clusters of erythroblasts or an equivalent number of erythroblasts

CFU-GM (colony-forming unit - granulocyte/ macrophage). Produces 20 or more granulocytes and macrophages

CFU-GEMM (colony-forming unit - granulocyte, erythrocyte, macrophage, megakaryocyte). Produces 20 or more cells including some from at least the first 2 types named

When leucocyte conditioned medium is used as a stimulant, colonies derived from CFU-E and mature BFU-E (BFU-E that produce colonies consisting of from 3 to 8 clusters of erythroblasts) should be scored after 10 to 12 days of incubation and after 18 days for the other types of colonies. Colony growth in methylcellulose cultures containing combinations of recombinant growth factors appears to proceed more rapidly and thus all categories of colonies can be scored after 14 to 16 days of incubation. Moreover under these latter conditions, some of the granulopoietic colonies will eventually become very large and therefore after 16 days may obscure the identification of some CFU-E-derived colonies. However, if cultures are scored too soon, some erythroid colonies may not yet be hemoglobinized and hence be mistakenly scored as granulopoietic.

Scoring or counting the number and types of colonies is best done using an inverted microscope that has been equipped with high quality flat field objectives and eye pieces to give object magnifications of approximately 20-30X, 80X and 150X. To



facilitate counting, each dish is first placed (with the lid on) within a 60 mm gridded tissue culture dish so that consecutive columns (or rows) within the 35 mm assay dish can be tracked. As shown in the picture above, the gridded 60 mm dish can be marked with a cross mark using a coloured felt pen to form four quadrants. By centering the culture dish on the intersection of the cross mark, it is possible to use the cross mark to subdivide the culture into halves or quarters to reduce the area scored when colonies are sufficiently numerous to make this desirable. However, to ensure that the portion of the dish being used for scoring is representative of the entire dish, it is necessary to first scan the entire dish at low magnification.

Considerable experience has shown that scoring is easier on the eyes when the culture dish is scanned vertically, i.e. by moving the culture dish up and down rather than sideways. Each assay should be set up at least in duplicate. The average number of colonies in a defined portion (or all) of the methylcellulose culture medium in a single petri dish is then used to calculate the average number of each type of progenitor detected in the original cell suspension assayed. This is based on the number of cells plated per unit volume of the final methylcellulose medium.

Cross comparisons of counts obtained by different members of staff on a regular basis are recommended to maintain consistent scoring criteria within a given laboratory. Care should also be taken to avoid disturbing the medium in the cultures at all times, as the methylcellulose is a viscous liquid, not a solid, and colony formation will be disrupted by tipping or other rough handling of the cultures.

Types of Colonies in Cultures of Normal Human Blood and Bone Marrow Cells

General

All hematopoietic colonies develop by single cells dividing and differentiating to eventually give rise to progeny that can be recognized morphologically. Under the conditions routinely used in colony assays, most progenitor cells appear to differentiate as they divide with the result that after a finite and predictable period of incubation, all cells have reached maturity and the colony will not increase in size any further. This is the time when each colony type is best evaluated as the mature cells produced will subsequently begin to lyse. This feature of colony development allows different stages of progenitor cell development to be distinguished according to the sizes of colony they ultimately generate, i.e. the bigger the colony (the greater the number of cells within the colony), the greater the proliferative capacity and the more primitive the original progenitor cell from which it arose. In the illustrations that follow, a time course study of each type of colony is shown to demonstrate the changes in appearance that occur as the colony reaches and then passes full maturity. In addition are shown different examples of colonies derived from the same progenitor class to illustrate the spectrum of different colony morphologies to be expected under 2 sets of "optimal" conditions (one with a recombinant cocktail (RC) of growth factors and one with Agar LCM).

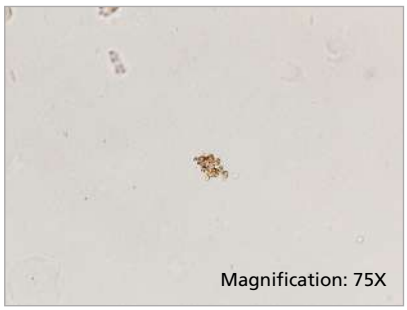
Erythroid Colonies

In methylcellulose assays, erythropoietic progenitor cells generate colonies that, when mature, may consist of varying numbers of discrete clusters of hemoglobinized erythroblasts. Normally these will not develop unless sufficient erythropoietin is present in the medium. However, the early stages of erythroid progenitor cells proliferation are stimulated by other factors and thus, even in the absence of erythropoietin, substantial numbers of cells may be produced from individual BFU-E before further growth is aborted. The final number of clusters of hemoglobinized erythroblasts present serves as a convenient yardstick for estimating the size of the colony and hence allows those derived from different stages of erythroid progenitor cell differentiation to be distinguished on a routine basis. However, the extent to which such clusters appear as distinct entities can also be readily influenced by changes in culture conditions. One of these is the final concentration of methylcellulose that is dissolved in the medium - the more viscous the medium, the greater the tendency of the clusters of erythroblasts to remain in one large grouping of cells and, conversely, the less viscous the medium, the greater the tendency of these clusters to fragment into larger numbers of smaller clusters. The types and concentrations of growth factors present is another parameter that strongly influences erythroid colony morphology. There is an obvious difference in the appearance of both erythroid colonies and multi-lineage colonies containing an erythroid component that are produced in methylcellulose media containing leukocyte conditioned media and recombinant growth factors. The extensive migratory capacity exhibited by developing erythropoietic precursors in the latter appears to be greatly reduced. As a result, distinct subcolonies or clusters of erythroblasts are usually less apparent or absent. Instead all of the cells within the colony stay together as a single large spherical or more irregular mass of cells, sometimes with a halo of erythroblasts around the periphery. It is therefore important to establish criteria for scoring erythroid colonies based on an estimate of the total number of cells in the colony as well as on the number of clusters making up the colony.

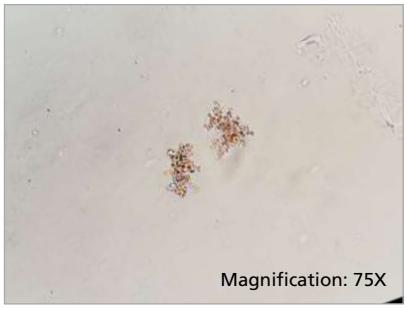
CFU-E

CFU-E (colony-forming unit - erythroid) is the term used to refer to those progenitor cells that give rise to the smallest and most rapidly maturing erythroid colonies. When mature, CFU-E-derived colonies consist of only one or two clusters containing up to a maximum of approximately 100 to 200 erythroblasts. Each cluster must contain a minimum of 8 erythroblasts to be scored. The erythroblasts are uniquely recognizable in methylcellulose by a distinctive reddish-orange hue due to their content of hemoglobin. Most colonies derived from CFU-E present in the original suspension plated will have reached maturity within 10 to 12 days in culture. After that time the cells in these colonies may begin to lyse. If scoring all colonies after 14 or more days of incubation, it should be kept in mind that the colonies may appear as containing lysed, brownish erythroblasts.

Agar LCM



Day 6



Day 6

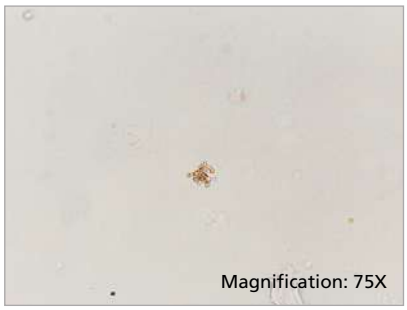
Time course study of colony formation from CFU-E

Note how the cells in the colonies are lysed by day 18. Also note the difference in size between the small mature erythroblasts and occasional larger cells (macrophages) in the background.

An undissolved piece of methylcellulose can be seen on the right hand side of the second row of pictures and in the bottom right corner of the fourth sequence.

RC

Day 6



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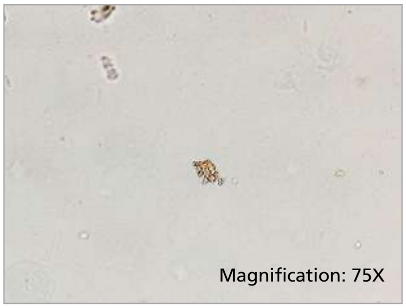

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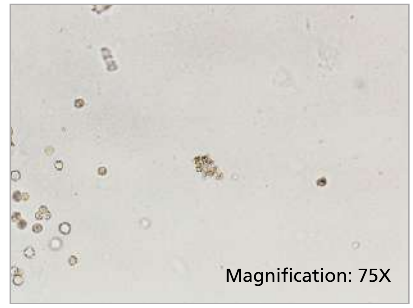

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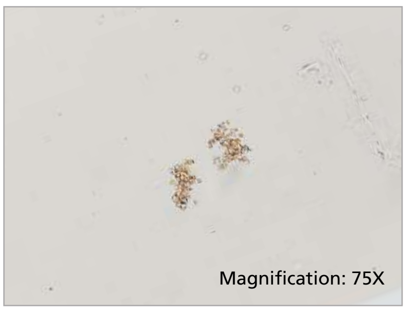

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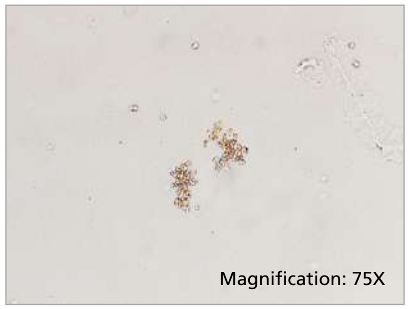

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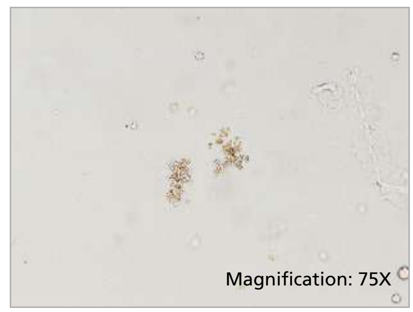
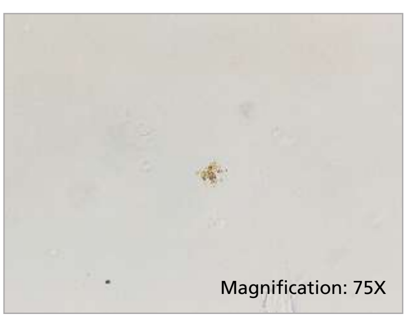


Day 18

Day 10

Day 14

Day 18



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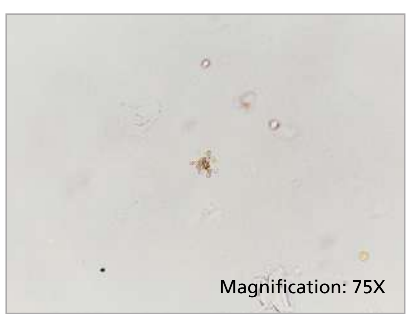

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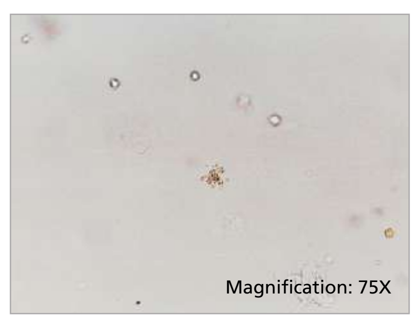
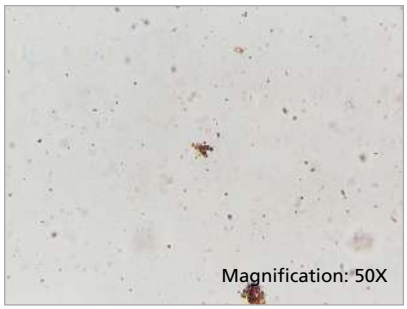


Day 18

Spectrum of colony sizes produced by CFU-E

Note that there is no noticeable difference in the appearance of colonies grown from CFU-E in either media.

Agar LCM



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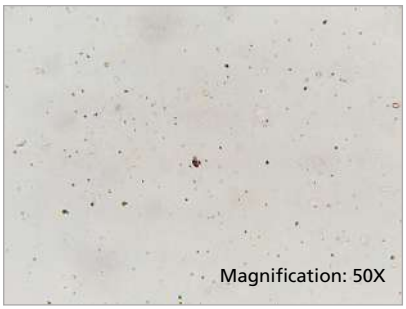

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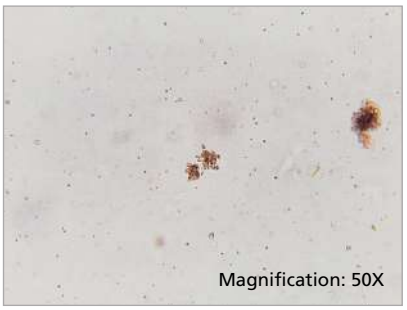

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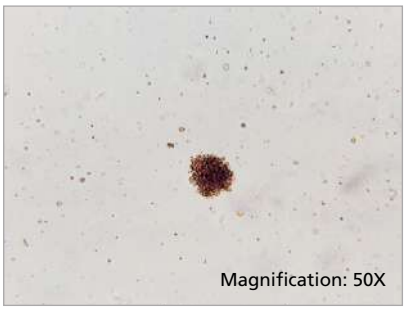
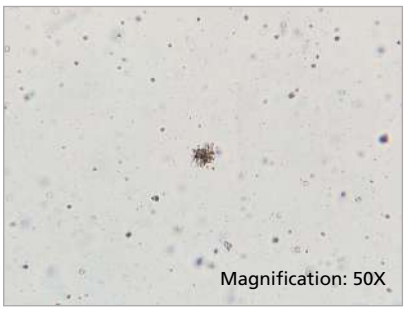


Day 10

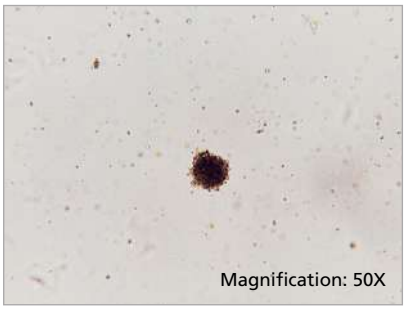
RC

Day 16



Day 16

Day 16



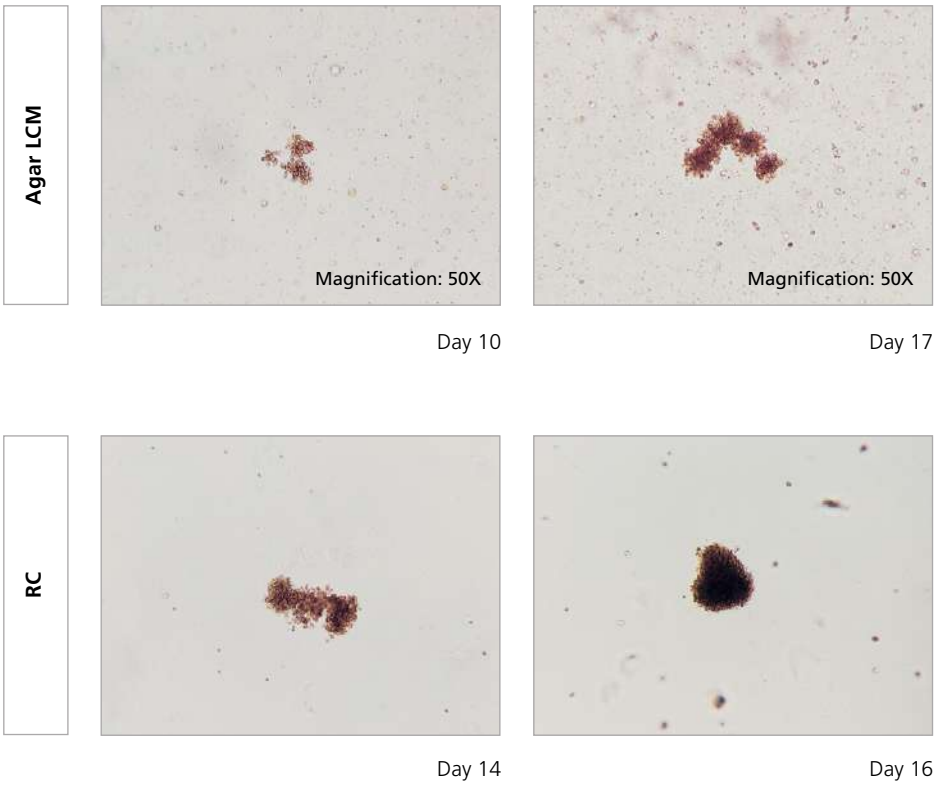
Day 16

BFU-E

The term BFU-E (burst-forming unit - erythroid) is used to denote a class of more primitive erythroid progenitor cells than CFU-E. The distinguishing property of the BFU-E is its greater proliferative capacity which enables it to give rise to larger, multi-clustered erythroid colonies than those produced from CFU-E. The term "burst" was coined to recognize the sudden appearance of hemoglobin in already large but previously colourless erythroid colonies due to the semi-synchronous pattern of erythroid cell differentiation. Erythroid bursts are also sometimes referred to as having a grapelike morphology. They may range in size from colonies consisting of 3 small clusters (or one large cluster) containing more than 200 erythroblasts to extremely large but pure erythroblast colonies containing 16 or more clusters and 10^4 or more individual cells. When scoring erythroid colonies grown in methylcellulose containing combinations of recombinant growth factors, it is usually more difficult to distinguish discrete clusters and hence assessing colony size must depend more often on an estimate of the number of cells in the colony.

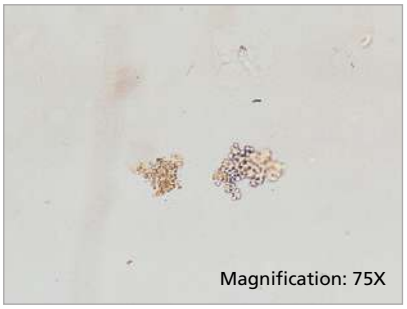
Mature BFU-E

Mature BFU-E is a term that is used to identify BFU-E that are the immediate precursors of CFU-E. They are defined by their ability to generate intermediate sized erythroid colonies that contain at least 3 but not more than 8 erythroblast clusters, i.e. corresponding to mature colonies of ~ 100 to 500 cells. Colonies produced by mature BFU-E are usually fully hemoglobinized within 10 to 12 days of incubation and may appear to contain lysed brownish erythroblasts when scored after day 16 or 18.



Spectrum of colony sizes produced by mature BFU-E

Agar LCM



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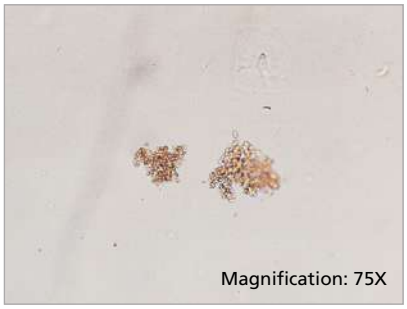

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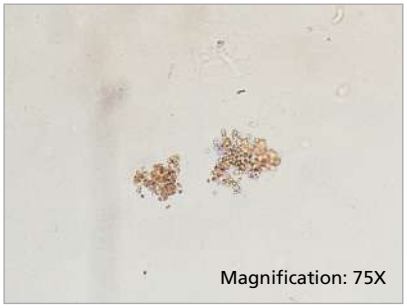

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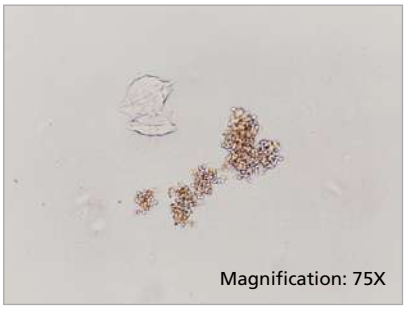

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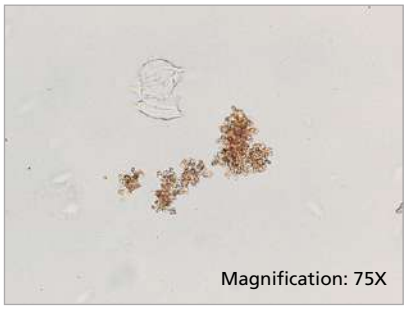

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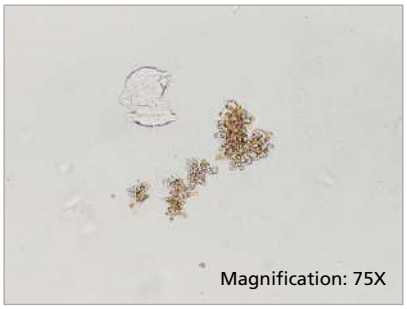

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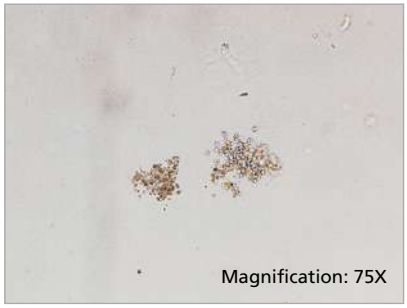

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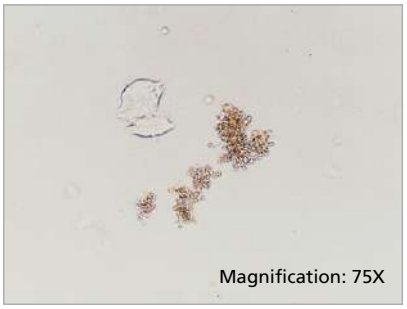


Day 18

Time course study of colony formation from mature BFU-E

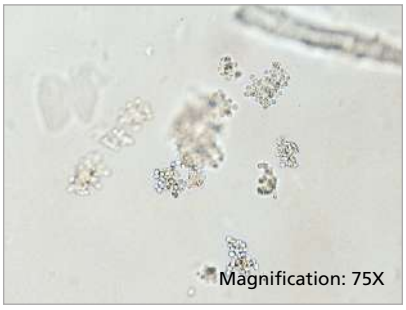
Note the piece of undissolved methylcellulose in the top left corner of the second sequence of pictures.

Primitive BFU-E

Primitive BFU-E are defined as those progenitors that give rise to 9 or more clusters of hemoglobinized erythroblasts (i.e. colonies consisting of > 500 cells). These colonies are best evaluated after 18 days of incubation in methylcellulose media containing leukocyte conditioned media or after 14 to 16 days when optimal concentrations of recombinant growth factors are used. At earlier times many of these colonies will not yet have begun to hemoglobinize and they may therefore be mistakenly confused with granulopoietic colonies. Occasionally, some erythroid colonies that will eventually achieve a very large size may not be fully hemoglobinized until after even 18 days.

Primitive BFU-E are sometimes further subdivided into those that generate 9 to 16 clusters of erythroblasts and those that generate more than 16 clusters of erythroblasts, to facilitate investigations of properties that change with very early erythroid progenitor cell differentiation.

Agar LCM



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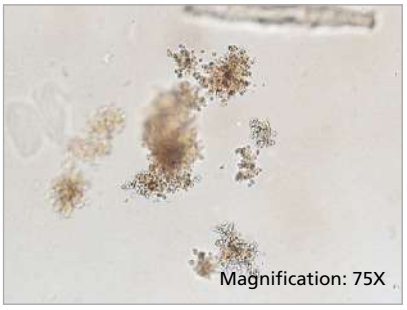

Day 14

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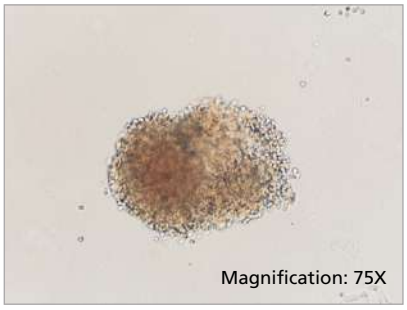

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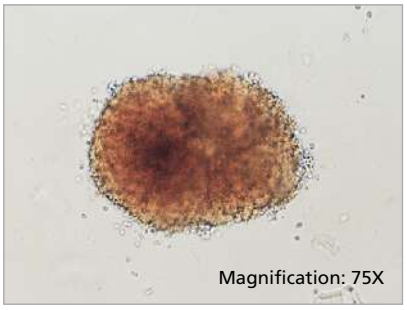

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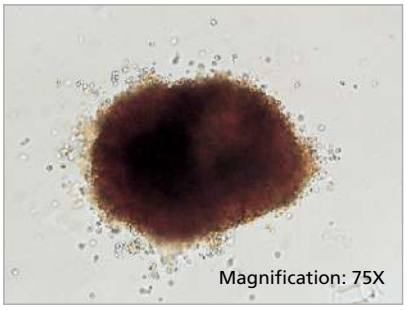
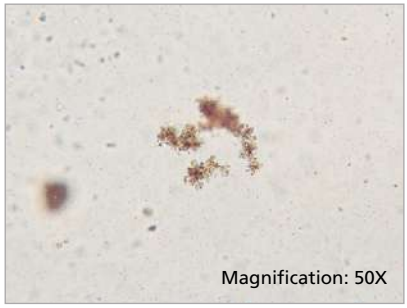


Day 14

Agar LCM



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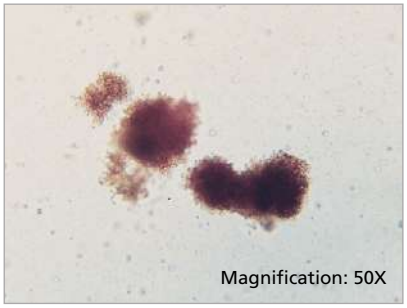
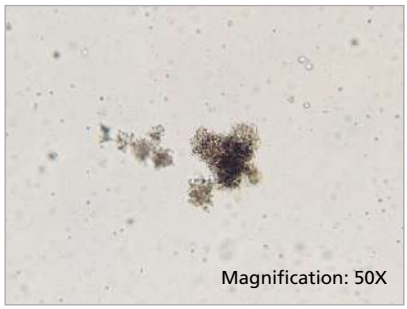


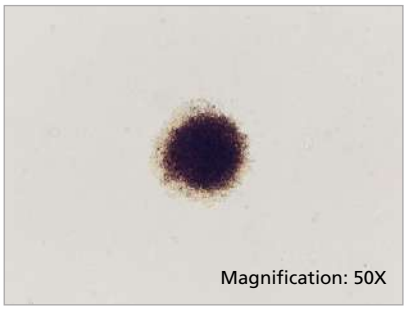
Day 17

Spectrum of colony morphologies produced by primitive BFU-E (that give rise to only 9 to 16 clusters of erythroblasts)

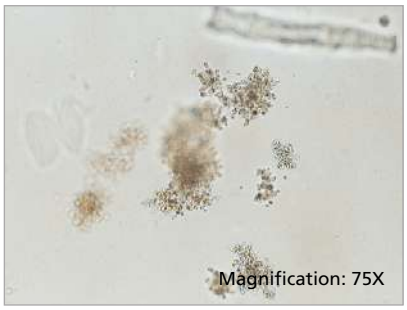
RC



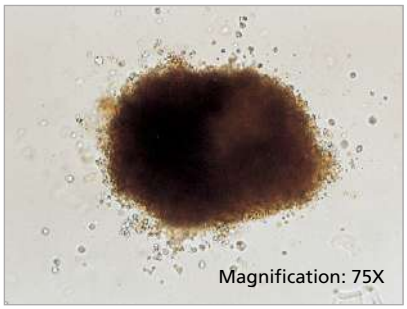
Day 14



Day 14



Day 18



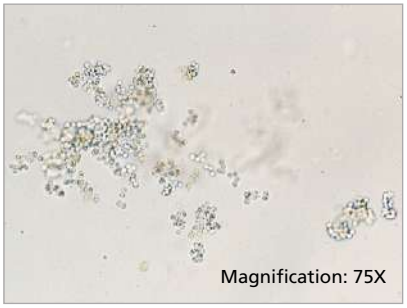
Day 18

Time course study of colony formation from primitive BFU-E (that produce 9 to 16 clusters of erythroblasts)

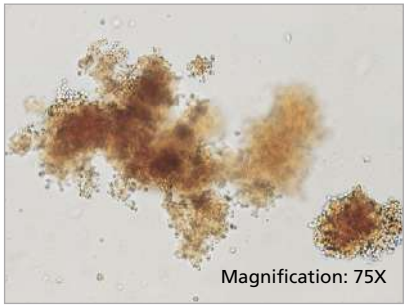
An undissolved piece of methylcellulose is visible in the top right corner of the first sequence.

The second sequence shows a typical large erythroid colony seen in RC-containing media where discrete erythroblast clusters tend not to form.

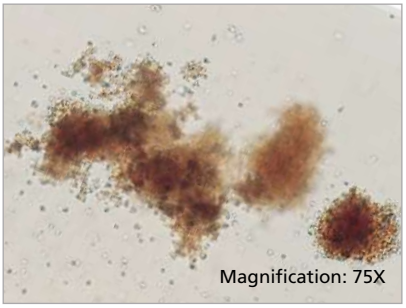
Agar LCM



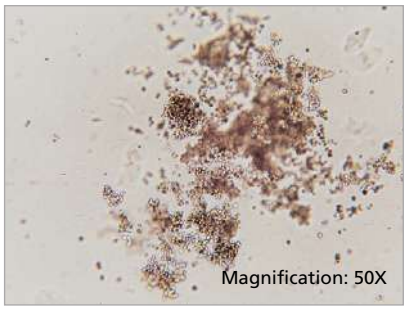
Day 6



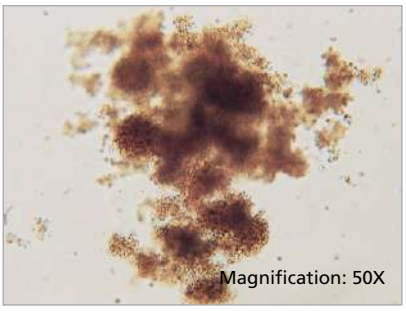
Day 10



Day 14

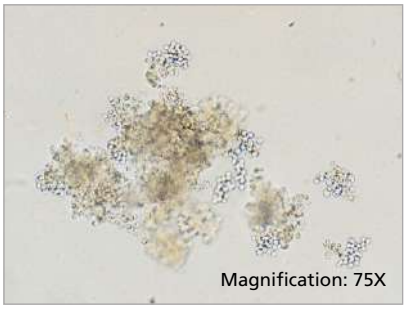


Day 10

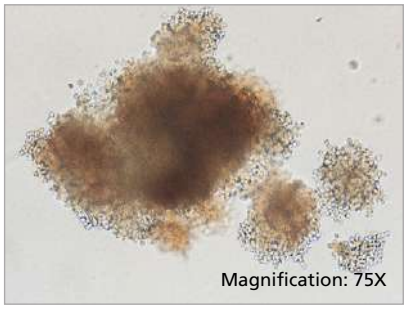


Day 14

RC



Day 8



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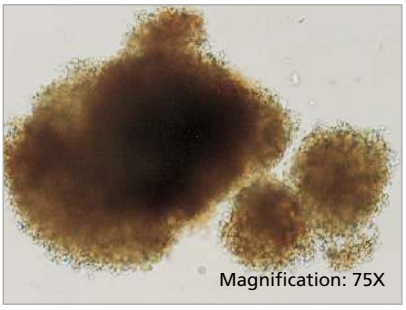

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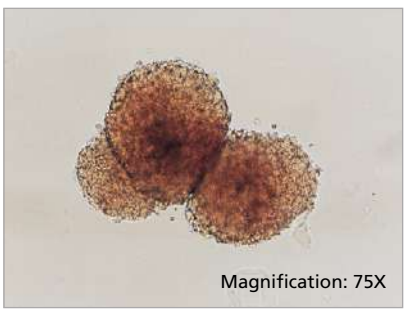

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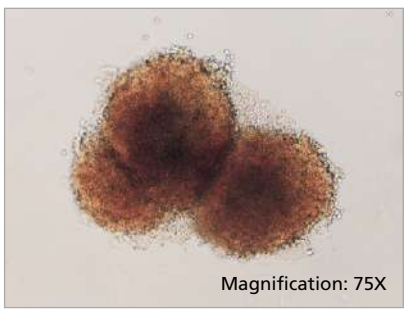

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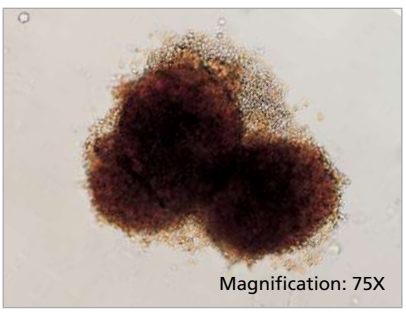
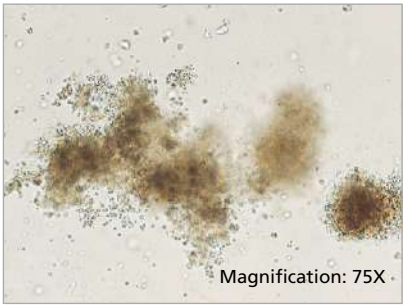
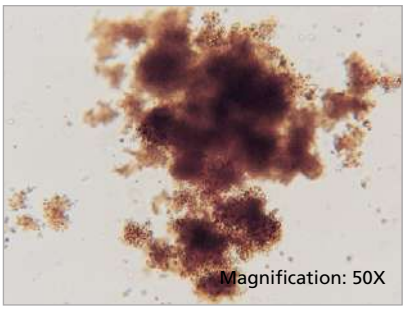


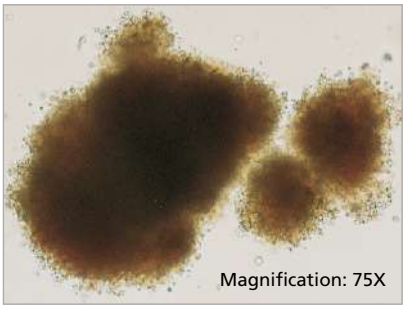
Day 14



Day 18



Day 18



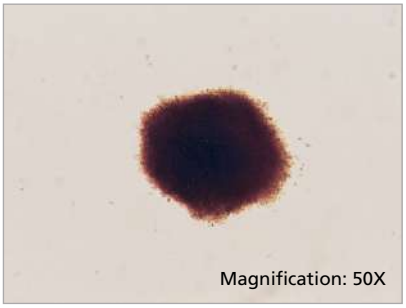
Day 18

Day 18

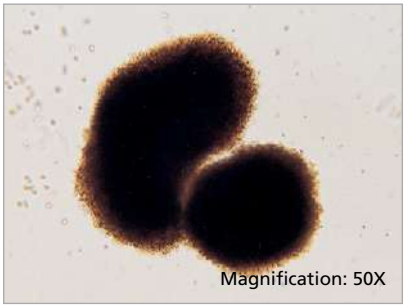
Time course study of colony formation from primitive BFU-E (that produce more than 16 clusters of erythroblasts)

Note the larger numbers of individual clusters in Agar LCM than in RC media.

RC



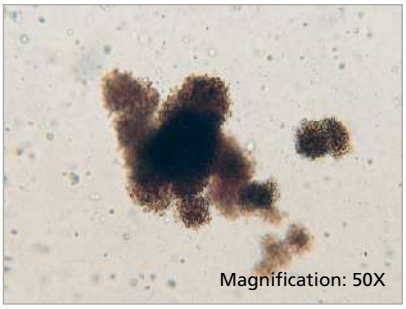
Day 14



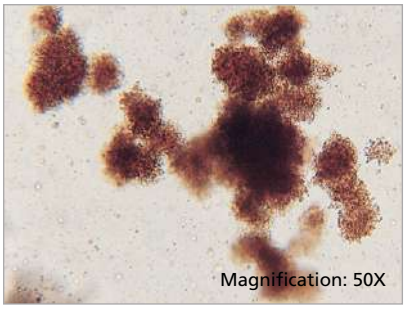
Day 14

Spectrum of colony morphologies produced by primitive BFU-E (that give rise to more than 16 clusters of erythroblasts)

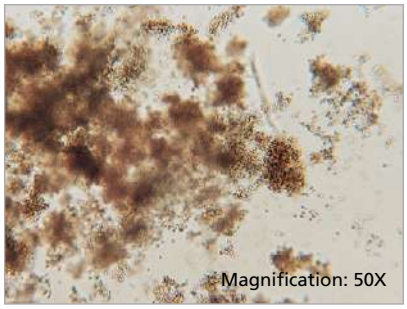
Agar LCM



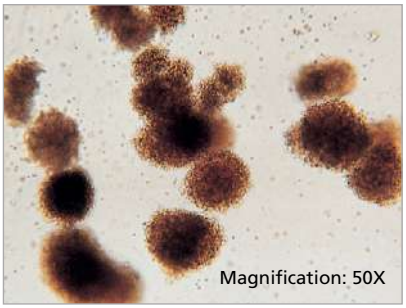
Day 16



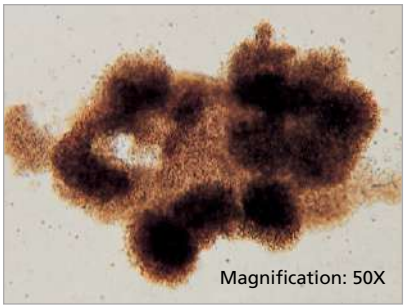
Day 17



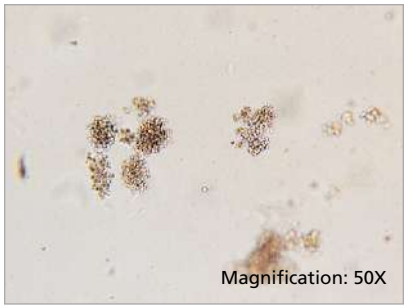
Day 18
Colony still not fully hemoglobinized



Day 14

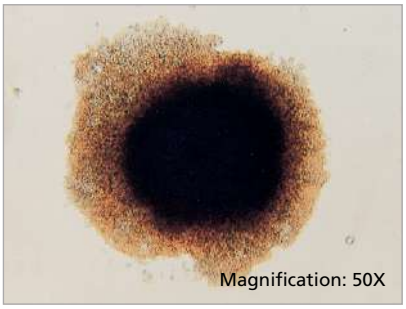


Day 14

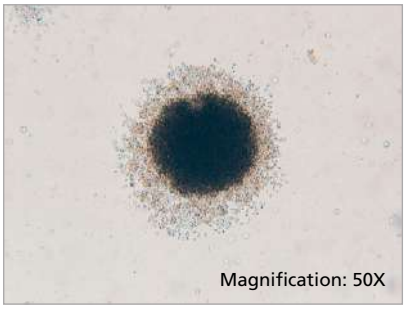


Day 14
Colony still not fully hemoglobinized

RC



Day 14



Day 16

Large erythroid colonies with "fried egg" morphology typically obtained with recombinant growth factor stimulation.

Granulopoietic Colonies

Clonogenic progenitors of granulocytes (CFU-G), macrophages (CFU-M), or both (CFU-GM) are defined on the basis of their ability to produce colonies containing a minimum of either 20 or 50 of these cells, depending on the scoring criteria developed in each laboratory.

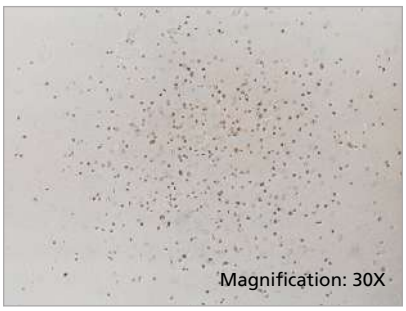
Granulopoietic progenitor cells were the first clonogenic hematopoietic cells to be grown in semi-solid media and were initially called CFU-C (colony-forming unit - culture) since it was not possible at the time to establish whether they had additional differentiative potentialities or not. When it later became evident that the vast majority of all CFU-C actually represented a population of progenitor cells restricted to the granulocyte and/or macrophage pathways of differentiation, the terms CFU-G, CFU-M and CFU-GM came into general use. Initially, most of the granulocyte colonies obtained in vitro, were actually colonies of neutrophils and/or macrophages, and only later were colonies of eosinophils and basophils (or mast cells) also recognized. Eosinophils and basophils are not typically seen admixed with macrophages and have a somewhat different appearance even in unstained preparations. When methylcellulose media containing leukocyte conditioned media are used to assay for both erythroid and granulopoietic progenitor cells in the same culture, it is best to wait until the cultures are 18 days old before scoring the granulocyte and macrophage colonies to avoid over-estimating their numbers by misassignment of early stage (non-hemoglobinized) erythroid colonies.

In general, neutrophil and macrophage colonies show a relatively homogeneous morphology, often with a more concentrated central core of cells surrounded by a less dense halo of cells. Neutrophils are small cells, macrophages tend to be larger, some becoming very large. Colonies of basophils are rarely found in routine assays. Colonies of eosinophils are more common, particularly in assays of progenitor cells found in the peripheral blood. Colonies of granulocytes and macrophages are difficult to subdivide reproducibly according to their size although, if a sufficiently high cut-off is used (e.g. 500 - 1000 cells), granulopoietic progenitor cells with different properties can be distinguished. In general when the methylcellulose contains defined recombinant growth factors, a larger proportion of the granulopoietic colonies produced will achieve a larger size than in methylcellulose cultures containing leukocyte conditioned medium.

Agar LCM

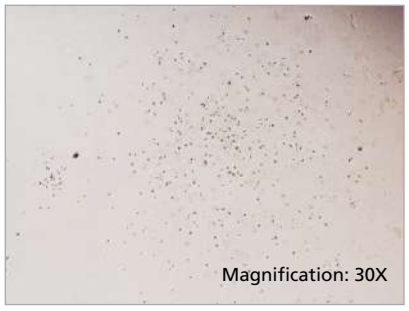
Day 6

Day 10



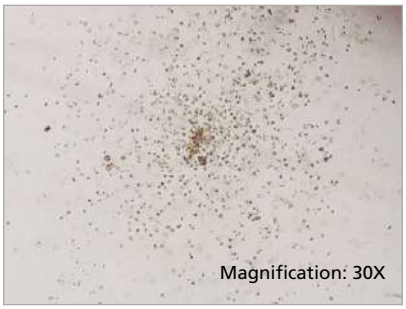
Day 14

RC



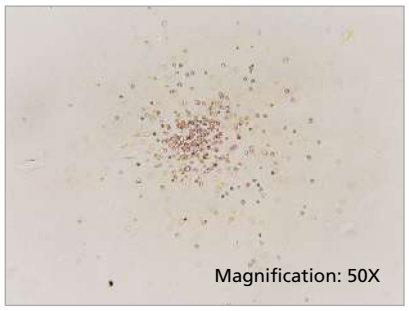
Day 6

Day 10

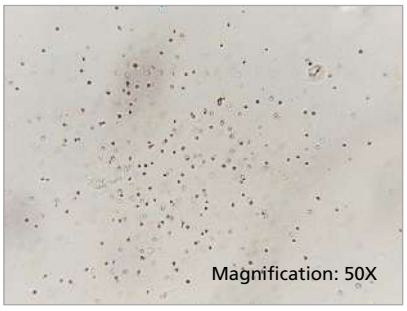


Day 14

Agar LCM



Day 6



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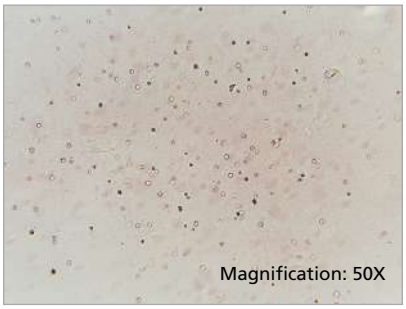

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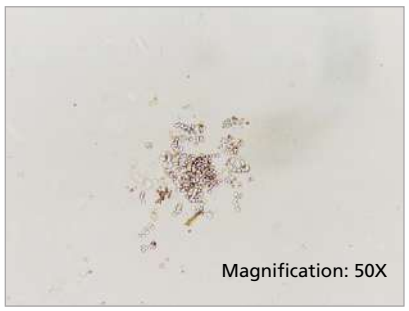

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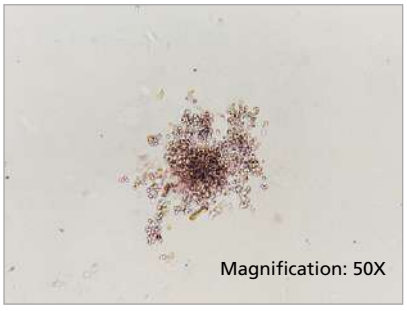
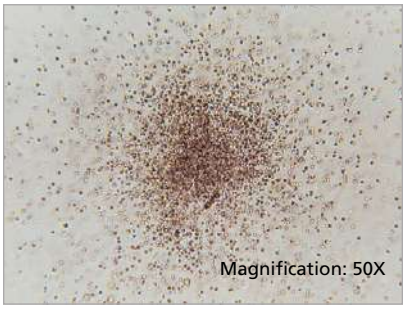
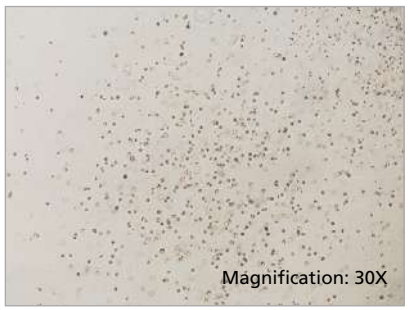


Day 10

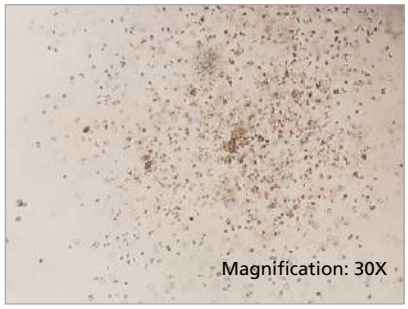


Day 14

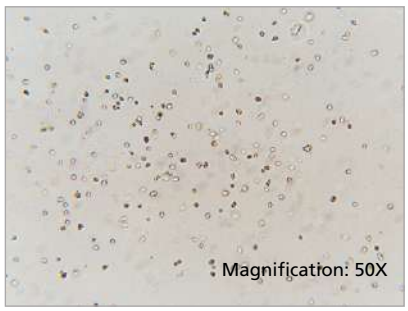
Time course study of colony formation from CFU-GM



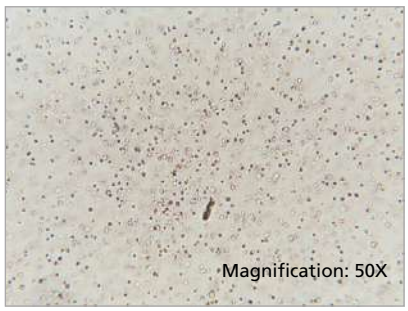
Day 18



Day 18

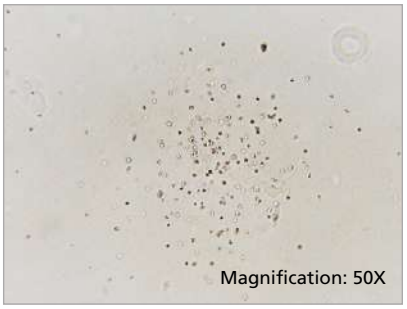


Day 18

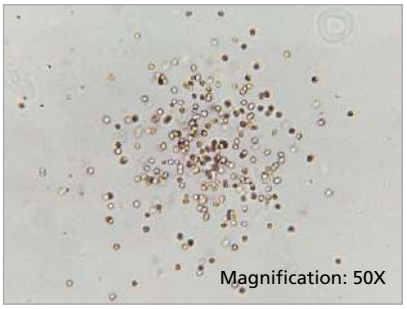


Day 18

Agar LCM



Day 6



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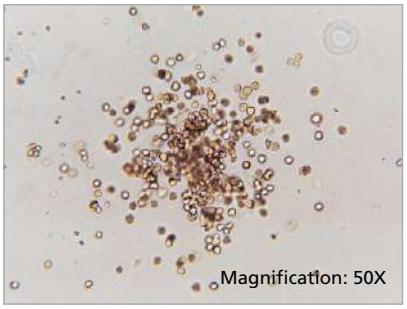

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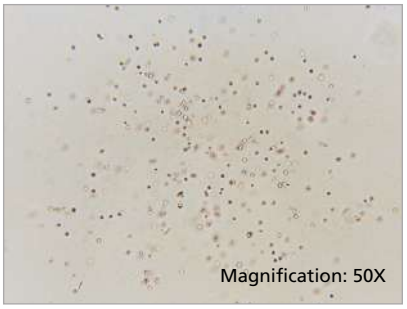
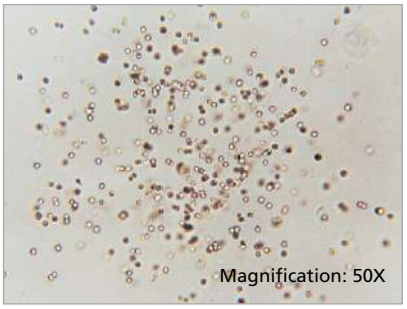
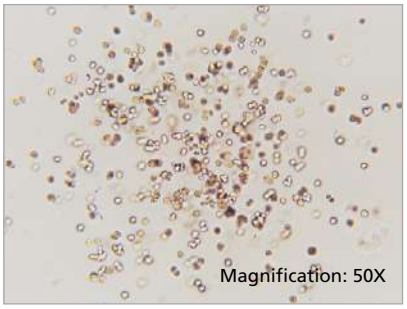


Day 6



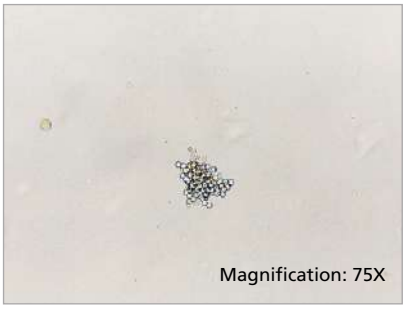
Day 10



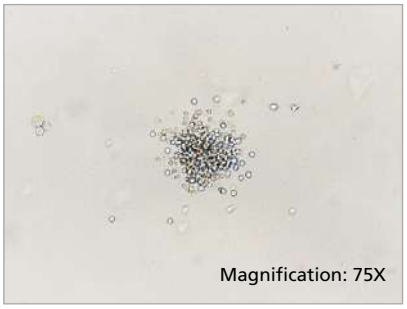
Day 14

Agar LCM

Day 6



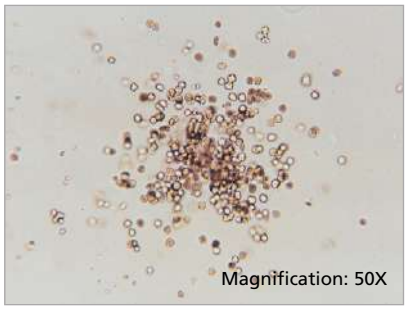
Day 10



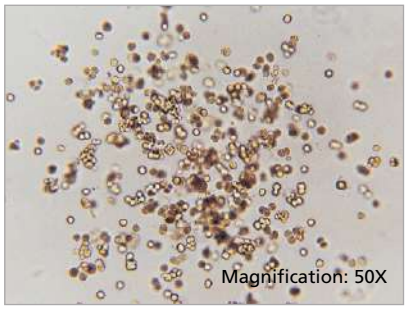
Day 14

Time course study of colony formation from CFU-M

Note how the macrophages produced increase in size over time even when the number per colony is no longer increasing.

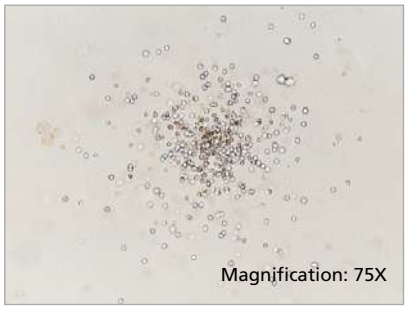


Day 18



Day 18

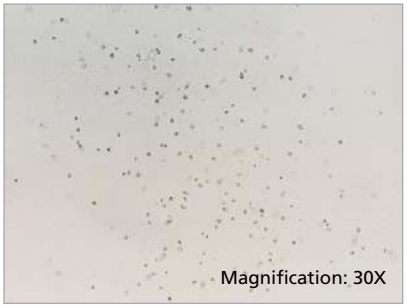
Time course study of formation of colonies of eosinophils



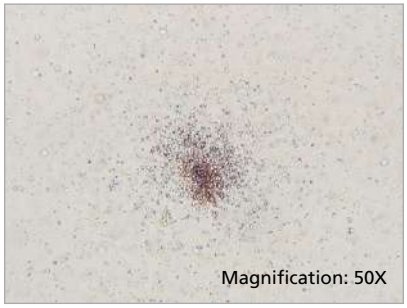
Day 18

Spectrum of colony morphologies produced by CFU-GM

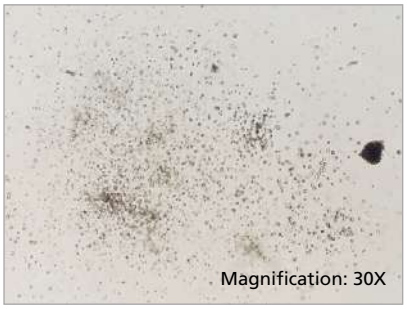
Agar LCM



Day 18



Day 18



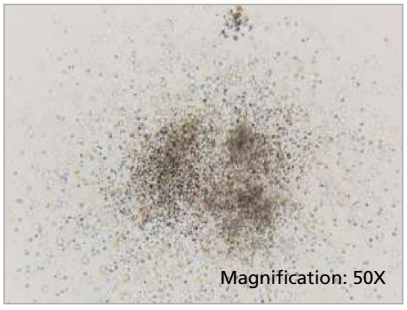
Day 18

Day 18

RC

Day 14

Day 14



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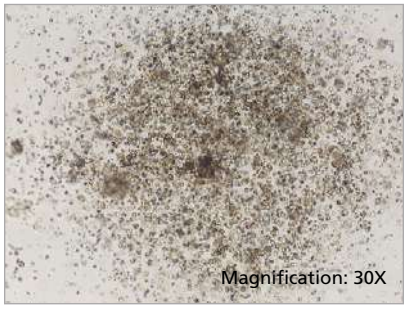
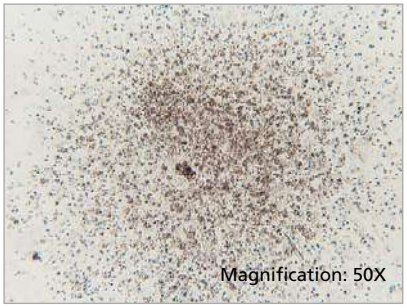


Day 16

Agar LCM



Day 20

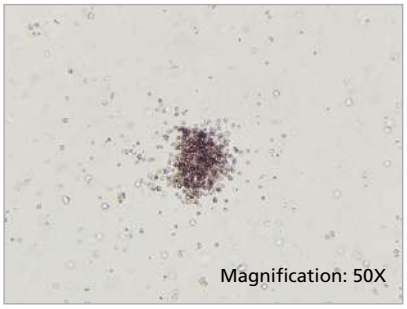
Day 20

Some CFU-GM from long-term cultures (LTC) will generate very large granulocyte-macrophage colonies.

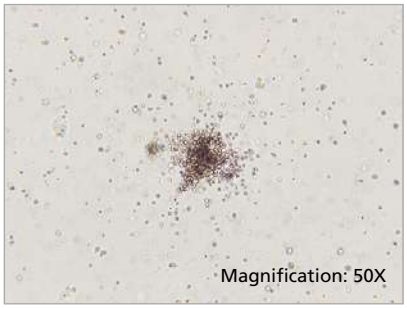
Day 20

Day 20

RC

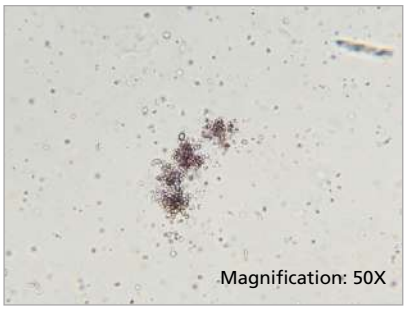


Day 17

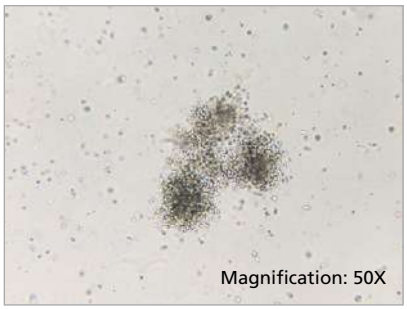


Day 17

Colonies of eosinophils



Day 17

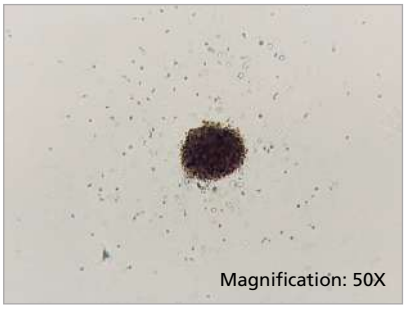


Day 10

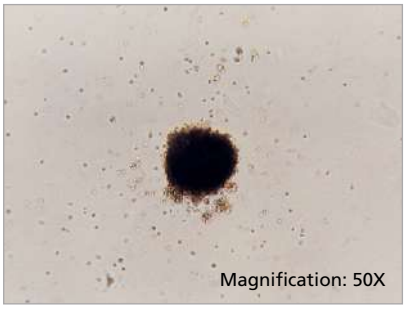
Multi-Lineage Colonies

CFU-GEMM (colony-forming unit - granulocyte, erythrocyte, macrophage, megakaryocyte) is the name given to progenitor cells that give rise to colonies containing multiple lineages of cells usually including erythroid cells. Such colonies are best evaluated after a minimum of 18 days of growth in media containing leukocyte conditioned media, or after 14 to 16 days when recombinant growth factors are used. In the latter case, extra care must be taken when scoring multi-lineage colonies. In some, small numbers of granulocytes, macrophages and/or megakaryocytes may appear around the periphery of a spherical mass of hemoglobinized erythroid cells. Multi-lineage colonies of this type can be mistakenly scored as pure erythroid colonies if not examined under high power.

RC



Day 6



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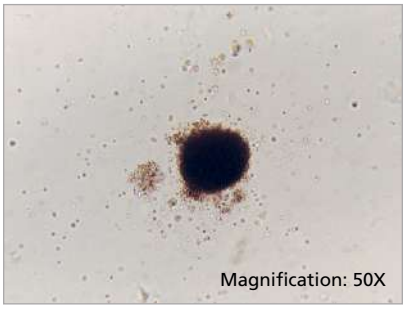
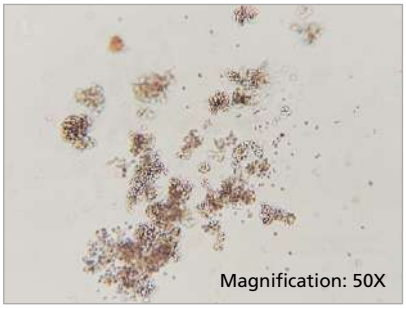


Day 14



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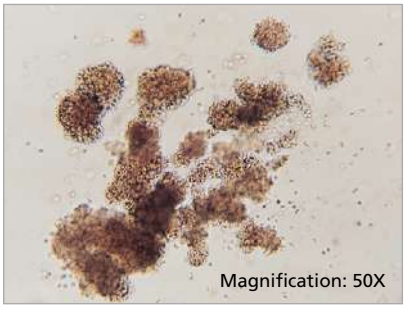

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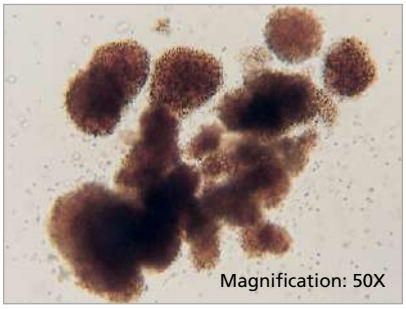

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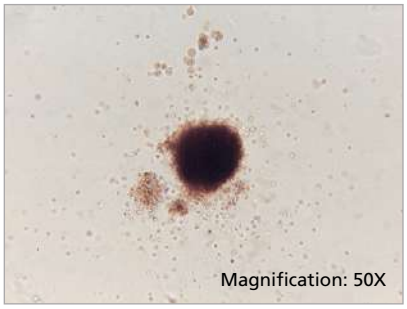
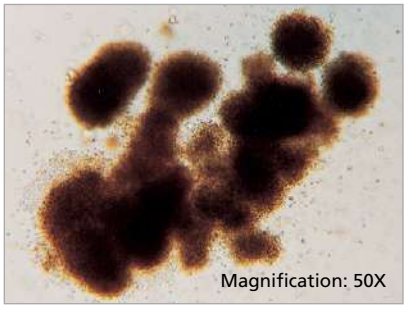


Day 18

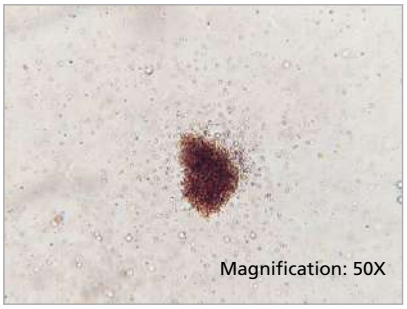


Day 18

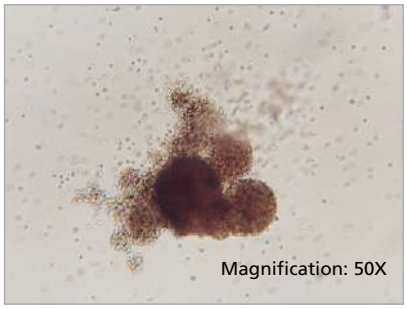
Time course study of multi-lineage colony formation from CFU-GEMM

Spectrum of different colony morphologies produced by CFU-GEMM

Agar LCM



Day 17



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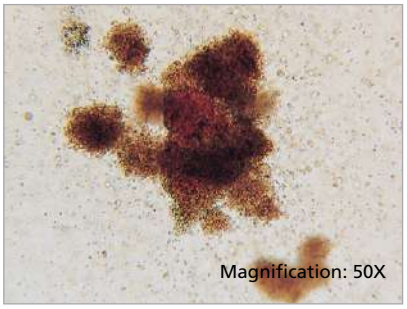
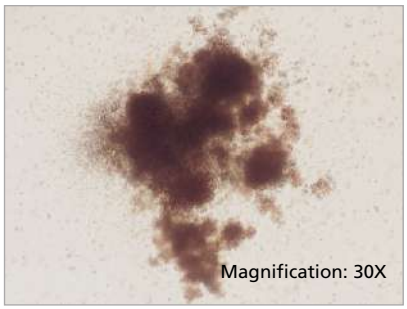
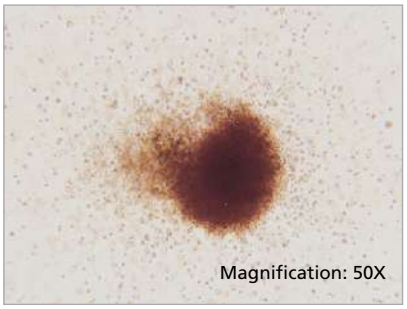


Day 17

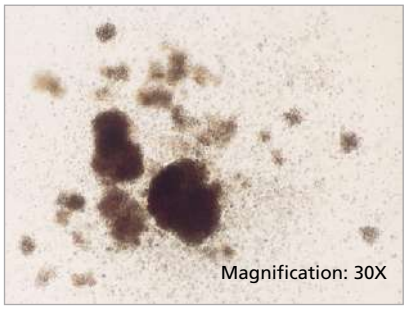


Day 18

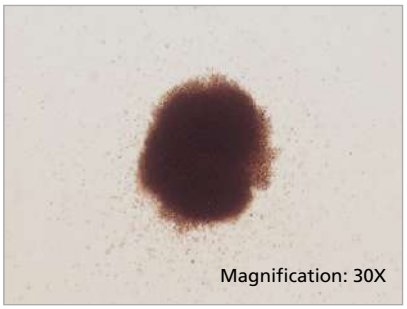
RC



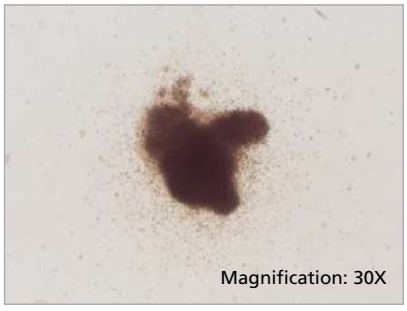
Day 14



Day 14

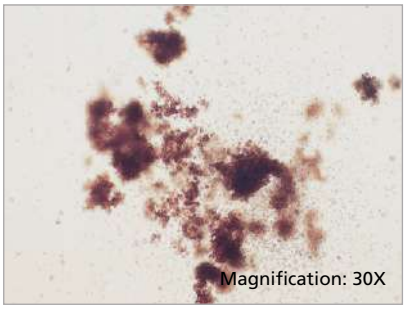


Day 14

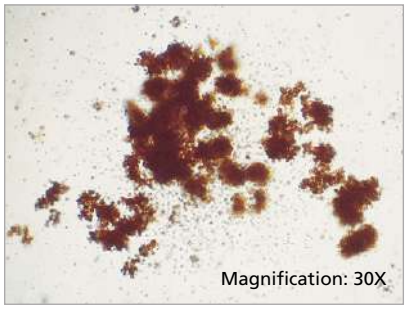


Day 16

Agar LCM



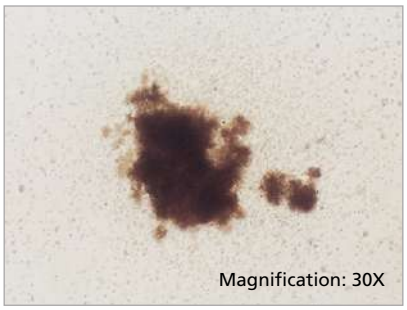
Day 20



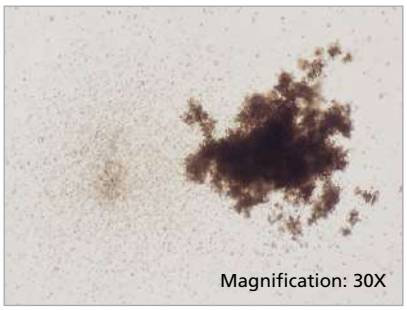
Day 20

Typical examples of multi-lineage colonies produced by CFU-GEMM from LTC.

Agar LCM



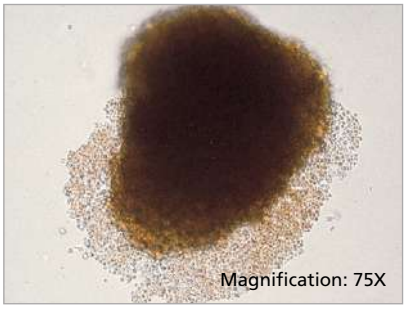
Day 18



Day 18

Colonies produced by CFU-GEMM

RC

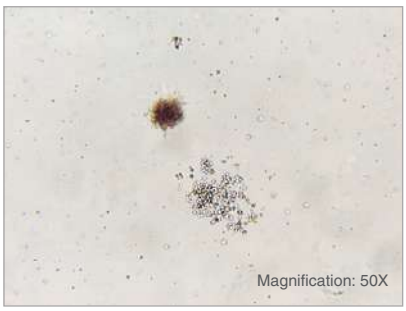


Day 18

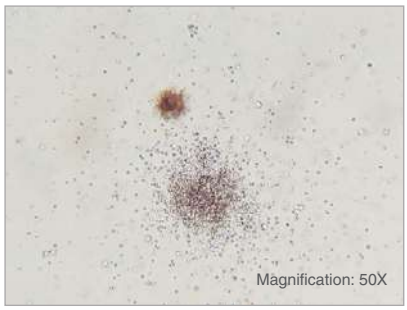
Day 18

Colonies produced by BFU-E with extensive halos of erythroblasts

Agar LCM



Day 10



Day 17

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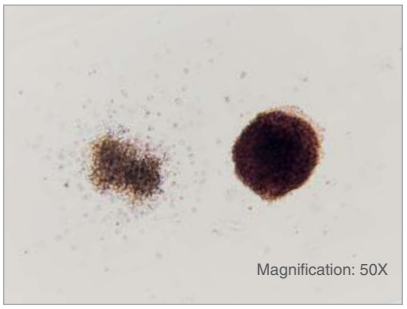
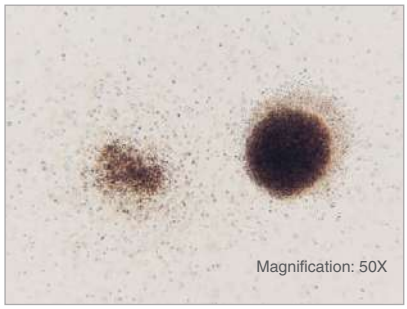


Day 10



Day 18

Colonies produced by adjacently plated BFU-E and CFU-GM (shown at both early and later timepoints)

Megakaryocyte Colonies

Megakaryocyte progenitor cells can also generate different sized colonies in methylcellulose. However, they are exquisitely sensitive to inhibition by TGF-ß (transforming growth factor-ß) which can be present in fetal calf serum at concentrations sufficient to prevent optimal megakaryocyte colony formation. More importantly, although many megakaryocytes have a large and uniquely translucent aspect, this is not a reliable way to identify all megakaryocyte colonies or to distinguish them from macrophage colonies. Megakaryocytes can, however, be recognized by specific immunohistochemical staining procedures. Because most pure megakaryocyte colonies contain less than 50 cells, their numbers rarely interfere significantly with counts obtained for CFU-M or CFU-GM. To assay for megakaryocyte progenitors (CFU-MK or BFU-MK), colony growth in serum-free agarose (rather than methylcellulose) containing suitable recombinant growth factors is recommended. Agarose cultures can then be fixed and stained in their entirety with antibodies for GPIIb/IIIa antigens to identify the megakaryocytic colonies. In view of these considerations, illustrations of living megakaryocyte colonies growing in methylcellulose cultures have not been included in this atlas.

Blast Colonies

Progenitor cells that are able to give rise to very large colonies (> 10^3 cells) will not produce mature progeny until after several cell generations. In addition, initiation of their growth may be more delayed than is characteristic of more differentiated progenitor types. Therefore, during the initial (2 week) period of growth of such colonies (which may span the first 2 to 3 weeks of culture), all of the cells they contain will, after removal and staining, be found to have a blast-like morphology. Moreover, if these cells are collected and redispersed in fresh methylcellulose medium, they will generate new daughter colonies at a high frequency. Since progenitor cells of such colonies are usually relatively rare by comparison to all other types of clonogenic cells, in most cell samples, blast colonies are not often detectable in routine assays. Normal blast colonies can, however, become more evident in preparations that are highly enriched for very primitive hematopoietic cells (for example in certain subpopulations of $CD34^+$ cells).

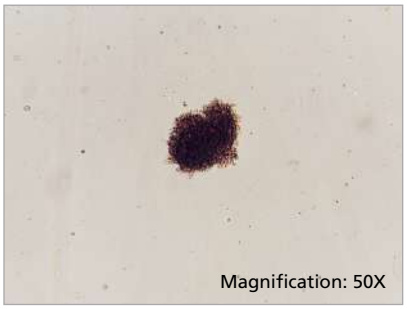
Colony Growth in Selected Disease States

Polycythemia Vera (PV)

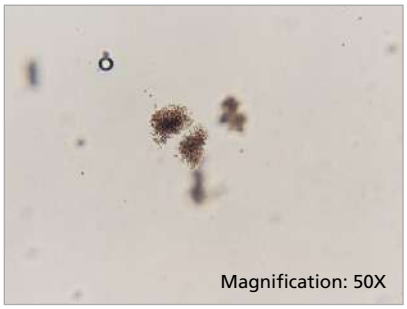
PV is a disorder in which a single abnormal clone comes to permanently dominate the hematopoietic system. In general, most differentiation processes are not affected, but whatever intrinsic, presumably genetically determined changes these clonal cells do acquire, they cause the production of too many red cells (hence the presence of a polycythemia). In vitro studies have shown that patients with PV have clonal erythroid progenitors that can differentiate into hemoglobin producing erythroblasts and this abnormality likely contributes to the overproduction of red blood cells that occurs in vivo. In addition this abnormality, because of its consistency in PV patients, can be used as the basis of a diagnostic test to discriminate between primary and secondary causes of erythrocytosis. Usually more than 10% of the CFU-E and BFU-E in PV patients will produce colonies of maturing erythroblasts in vitro in the absence of erythropoietin. These colonies will sometimes appear as well hemoglobinized as the erythroid colonies produced in the presence of erythropoietin, although usually their size and degree of maturation is not as well developed as is seen in parallel cultures to which erythropoietin has been added. Because this abnormal "erythropoietin-independence" persists, even after years of treatment, it is useful in establishing the correct diagnosis in PV patients regardless of whether they have a normal hematocrit as a result of previous treatment, bleeding, or iron deficiency. Also, because erythropoietin-independence is an intrinsic property of the neoplastic erythroid progenitor, it is manifested by such cells in the blood as well as in the marrow.

In other myeloproliferative diseases, such as chronic myeloid leukemia (CML), myelofibrosis and essential thrombocytosis, erythropoietin-independent colonies may also be detected, although not as consistently. Moreover, the erythroid colonies produced by these progenitor cells usually do not reach the same degree of hemoglobinization in the absence of erythropoietin as is seen with erythropoietin-independent progenitors from PV patients.

Methylcellulose media containing recombinant growth factors minus erythropoietin is not recommended for assessment of erythropoietin-independent growth due to the breakthrough erythroid growth which may occasionally be seen with the high concentrations of growth factors in the cocktails usually employed to achieve maximal numbers of normal erythroid colonies.



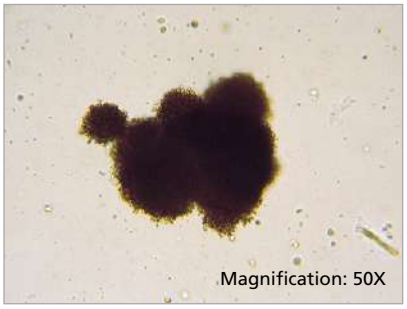
With EPO



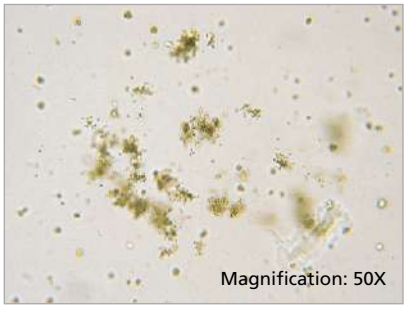
Without EPO

Erythropoietin-independent colony growth

Comparison of erythroid colonies produced after 16 days by progenitors from a PV patient grown in the presence and absence of erythropoietin.



With EPO



Without EPO

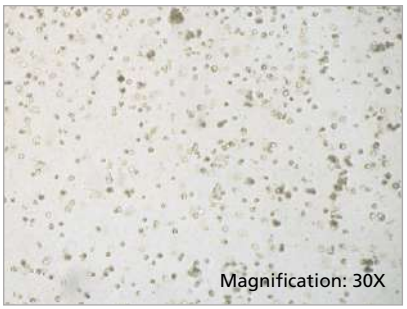
Comparison of erythroid colonies produced after 16 days by progenitors from a CML patient grown in the presence and absence of erythropoietin.

Chronic Myeloid Leukemia (CML)

In addition to erythropoietin-independent CFU-E and BFU-E, which are detectable in approximately 50% of blood and marrow samples from patients with CML (see section on PV, page 31), there is usually a marked increase in both the frequency and number of all types of clonogenic progenitors in CML blood. In general, the extent of this increase is related (exponentially) to the WBC count, although this varies enormously between individual CML patients. Thus with each doubling of the WBC count, on average, a four-fold increase in total progenitors per ml of blood can be anticipated and the frequency of progenitor cells, relative to other types of light density cells will also increase as the WBC increases. To ensure that the number of colonies to be produced in a given assay (of fixed volume) is neither excessive nor insufficient, it is necessary to plate cells from such samples at the usual and a lower cell concentration. Also typical of CML cultures is the presence of an increased background of macrophages. When the disease has evolved into blast crisis or sometimes already when it is in an accelerated phase, the presence of numerous, small, poorly differentiated "blast colonies" similar to those found in assays of cells from AML patients may occasionally be seen (see below).

Examples of an increased background of macrophages in CML cell cultures

Agar LCM



Day 18

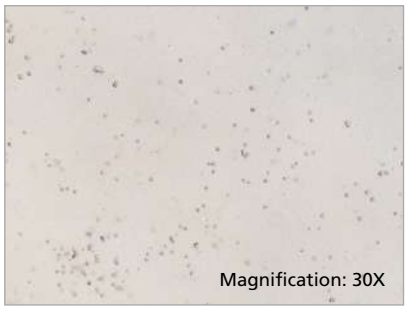
RC

Day 16

Myelodysplastic Syndromes (MDS) And Acute Myeloid Leukemia (AML)

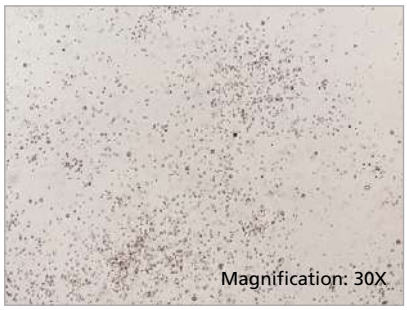
For the majority of patients with myelodysplastic disorders and patients with untreated or relapsed AML, in vitro colony assays of marrow or blood cells obtained at diagnosis reveal reduced or absent numbers of normal CFU-E, BFU-E, CFU-GM and CFU-GEMM. In addition, some patients presenting with a myelodysplastic marrow and the majority of patients with AML are found to have a population of abnormal progenitor cells in the marrow and blood. These form colonies or smaller clusters of cells that do not mature and resemble the leukemic blasts that are found in vivo in these patients. Such colonies are typically smaller and more numerous than would be expected for normal progenitor cells in assays of diagnostic material. However, exceptions to this are common. For example, in some cases no evidence of colony or even cluster formation is seen. In others, occasional normal appearing granulopoietic and/or erythroid colonies may be seen together with variable numbers of "leukemic" blast colonies. Following remission induction, the leukemic blast colony progenitor cells decline to undetectable levels and normal erythroid and myeloid clonogenic progenitor cells reappear.

Blast background



Blast background

Blast clusters



Blast colonies

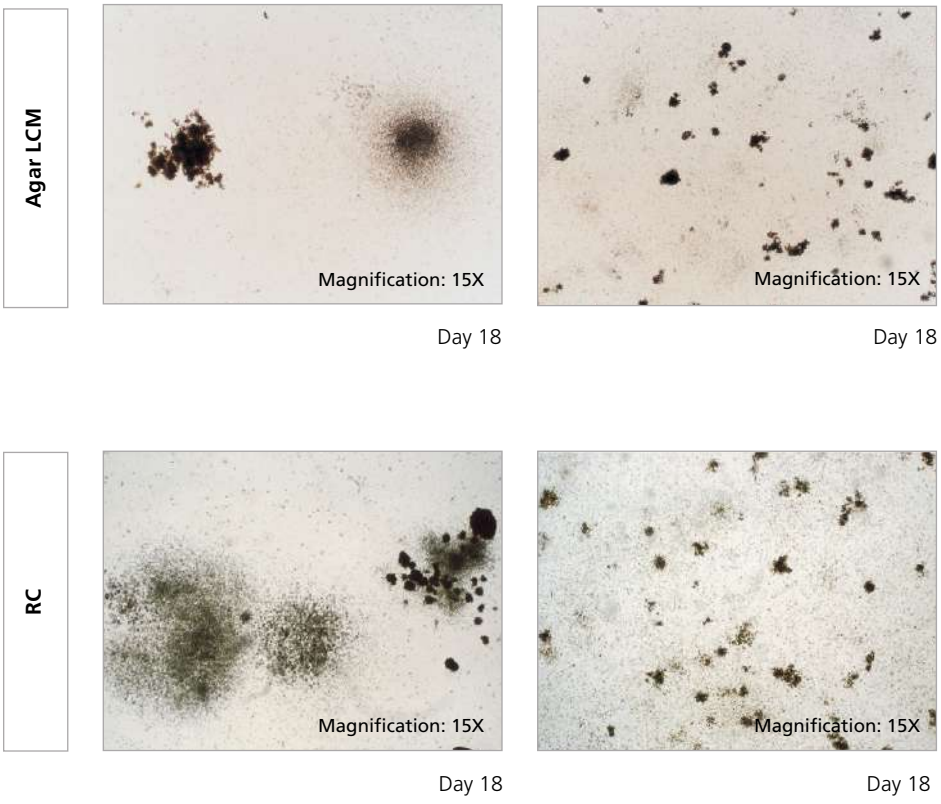
Typical examples of 16 day old cultures of AML cells illustrating a background of blast cells, small clusters of blasts and larger blast colonies

Notes on Colony Formation (Trouble Spots)

Overplating

If too many clonogenic progenitor cells are plated, overall colony growth is inhibited. The result is a reduction primarily in the final size that the colonies achieve. Such cultures are also difficult to score because the colonies are so close together. However, some progenitor cells may even be prevented from generating sufficient progeny to form a detectable colony. Erythroid colonies are particularly sensitive to excess numbers of granulopoietic colonies developing in the same culture since the pH of the culture may fall below 7.0. Hemoglobin synthesis is greatly reduced when the pH of the culture medium decreases below 7.0 and this can severely compromise the identification of erythroid colonies. In general, assays should be set up so that less than 100 colonies (total) and ideally approximately 50 colonies (total) are obtained in a 1 mL culture volume. Since the exact frequency of clonogenic cells is frequently not known, it is often useful to plate each assay at different cell concentrations.

Problems of overplating are increased when assays containing recombinant growth factors are used because, under these conditions, some of the colonies can become very large and their ability to inhibit the growth of one another is even greater. In addition, the number of clonogenic progenitor cells detectable in a given cell suspension is approximately 2-fold higher than what was typically seen with concentrations of leukocyte conditioned media that were previously thought to be optimal. Accordingly, in assays containing recombinant growth factors the number of cells plated should be correspondingly lower. Examples of where clonogenic progenitor numbers are frequently elevated (relative to other cell types) are in CML, in leukapheresis harvests of mobilized progenitor cells, and in samples obtained during the early period of hematopoietic recovery after combination chemotherapy treatment of AML or other malignancies.



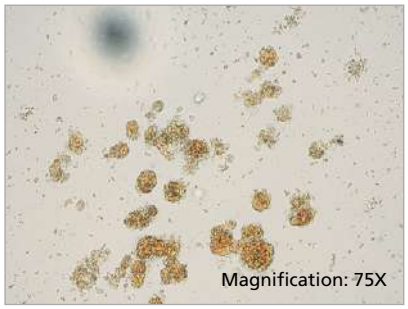
Inhibitory effect of overplating on colony formation

Each pair of pictures shows the same cells plated at a suitable concentration (on the left) and at a 10X higher concentration (on the right).

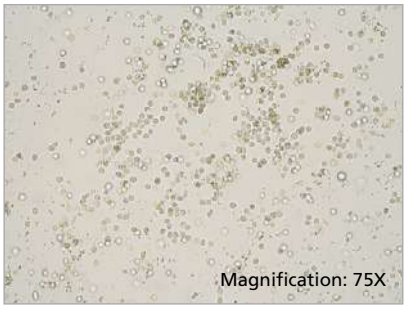
Inadequate viscosity

If the concentration of methylcellulose is too low, the viscosity of the culture will not be adequate to keep the cells localized in discrete single colonies. Instead, even the most controlled handling of the dish will cause colonies to break apart into fragmented clusters or to be dispersed as "streams" of cells. If this occurs, identification of individual colonies becomes impossible. If, on the other hand, the methylcellulose medium is too viscous, cell movement will be inhibited and colony growth, itself, is inhibited. The result is that colonies of different sizes, particularly those containing erythroid cells, are more difficult to discriminate and it will appear that the proportion of progenitor cells able to generate the largest type of colonies is reduced.

Agar LCM



BFU-E Day 18



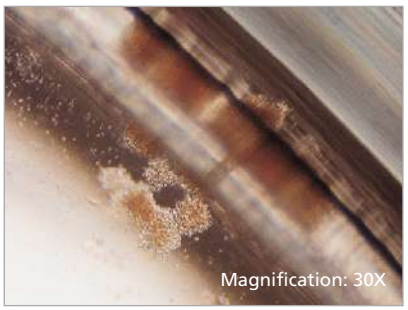
CFU-GM Day 18

Examples of overdiluted methylcellulose

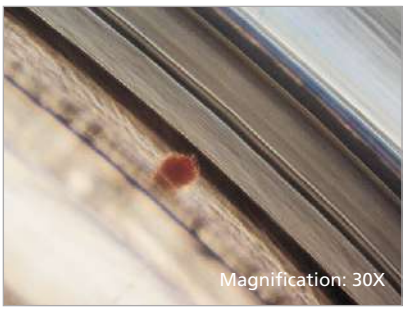
Colonies on the edge of the culture dish

It is important to remember that a disproportionate volume of the culture will be located at the edge of the dish due to the formation of a meniscus. Extra care is required to visualize the colonies contained in this region. This necessitates greater focusing of the objective since the colonies in this region of the culture are distributed over a greater depth of field. The following examples illustrate this.

Agar LCM



Day 14



Day 14

Examples of the appearance of colonies that form on the edge of the culture

Suggested Reading

Bernstein ID, Andrew RG, Zsebo KM. Recombinant human stem cell factor enhances the formation of colonies by $CD34^+$ and $CD34^+lin^-$ cells, and the generation of colony-forming cell progeny from $CD34^+lin^-$ cells cultured with interleukin-3, granulocyte colony-stimulating factor; or granulocyte-macrophage colony-stimulating factor. Blood 1991; 77:2316-21.

Eaves CJ. Myelopoiesis (chapter 4) In: Leukemia, 6th ed, Henderson, Lister, Greaves, Saunders Co, London, 1995

Eaves CJ, Eaves AC. Erythropoietin (Ep) dose-response curves for three classes of erythroid progenitors in normal human marrow and in patients with polycythemia vera. Blood 1978; 52:1196-210.

Golde DW, Takaku F. Hematopoietic Stem Cells. New York; M Dekker Inc., 1985.

McCulloch EA (ed.) Cell Culture Techniques. Clinics in Haematology. Vol 13. Saunders Co, London, 1984.

McNiece IK, Langley KE, Zsebo KM. Recombinant human stem cell factor synergizes with GM-CSF, IL-3 and Epo to stimulate human progenitor cells of the myeloid and erythroid lineages. Experimental Hematology 1991; 19:226-31.

Metcalf D. The Hemopoietic Colony Stimulating Factors. Elsevier, Amsterdam, 1984.

Sutherland HJ, Eaves AC, Eaves CJ. Quantitative assays for human hematopoietic progenitor cells. In: Bone Marrow Processing and Purging: A Practical Guide, ed AP Gee, CRC Press Inc., Boca Raton, pp. 155-71, 1991.

Testa NG, Molineux G (eds.) Haemopoiesis: A practical approach. Oxford University Press, Inc. New York. 1993.

Abbreviations

Agar LCM	agar-stimulated leukocyte conditioned media
AML	acute myeloid leukemia
BFU	burst-forming unit - erythroid
CFU	colony-forming units
CFU-C	colony-forming unit - culture
CFU-E	colony-forming unit - erythroid
CFU-G	colony-forming unit - granulocyte
CFU-GEMM	colony-forming unit - granulocyte, erythroid, macrophage, megakaryocyte
CFU-M	colony-forming unit - macrophage
CML	chronic myeloid leukemia
MDS	myelodysplastic syndromes
PV	polycythemia vera
RC	cocktail of recombinant growth factors
LTC	long-term culture
WBC	white blood cell

MethoCult™ Formulations and Plating Concentrations

Formulations of Human MethoCult™ Products

Methocult™ Product	Catalog #	Format	Components						
			FBS	BSA	Insulin + Transferrin	2-ME	Growth Factors		
							EPO	SCF, GM-CSF, IL-3	Other
H4034 Optimum	04034 04044 04064	100 mL 24 x 3 mL Starter Kit	•	•		•	•	•	G-CSF
H4035 Optimum without EPO	04035	100 mL	•	•		•		•	G-CSF
H4434 Classic	04434 04444 04464	100 mL 24 x 3 mL Starter Kit	•	•		•	•	•	
H4534 Classic without EPO	04534 04544 04564	100 mL 24 x 3 mL Starter Kit	•	•		•		•	
H4435 Enriched	04435 04445	100 mL 24 x 3 mL	•	•		•	•	•	IL-6, G-CSF
H4535 Enriched without EPO	04535 04545	100 mL 24 x 3 mL	•	•		•		•	IL-6, G-CSF
Express with EPO	04437 04447	100 mL 24 x 3 mL	•	•		•	•	Proprietary Formulation	
SF H4436	04436	100 mL		•	•	•	•	•	IL-6, G-CSF
SF H4536	04536	100 mL		•	•	•		•	IL-6, G-CSF
SF H4636	04636	100 mL		•	•	•	•	•	IL-6, G-CSF
H4431	04431	100 mL	•	•		•	•		Agar-LCM
H4531	04531	100 mL	•	•		•			Agar-LCM
H4100	04100	40 mL							
SF H4236	04236	80 mL		•	•	•			
H4330	04330	90 mL	•	•		•	•		
H4230	04230	80 mL	•	•		•			

2-ME = Mercaptoethanol; Agar-LCM = Agar-Leukocyte Conditioned Medium; PHA-LCM = Phytohemagglutinin-Leukocyte Conditioned Medium.

Recommended Plating Concentrations for CFU Assays of Human Cells

Cell Source	Cells Per 35 mm Dish
Bone Marrow - Ammonium Chloride Treated	2×10^4 - 1×10^5
Bone Marrow - Mononuclear Cells*	$1 - 5 \times 10^4$
Cord Blood - Mononuclear Cells*	5×10^3 - 2×10^4
Normal Peripheral Blood - Mononuclear Cells*	$1 - 4 \times 10^5$
Mobilized Peripheral Blood	$1 - 5 \times 10^4$
Lineage-Depleted $CD34^+$ Cell Enriched BM, CB, MPB	$0.5 - 2 \times 10^3$**
Purified $CD34^+$ cells (BM, CB, MPB)	$0.15 - 1 \times 10^3$**

BM = Bone Marrow; CB = Cord Blood; MPB = Mobilized Peripheral Blood.
*Mononuclear cells (MNCs) isolated by density-based cell separation (light density, 1.077 g/mL).
**Dependent on $CD34^+$ cell purity. Generally, 10 - 20% of $CD34^+$ cells form colonies.

HUMAN HEMATOPOIETIC COLONIES

Atlas of Human Hematopoietic Colonies

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