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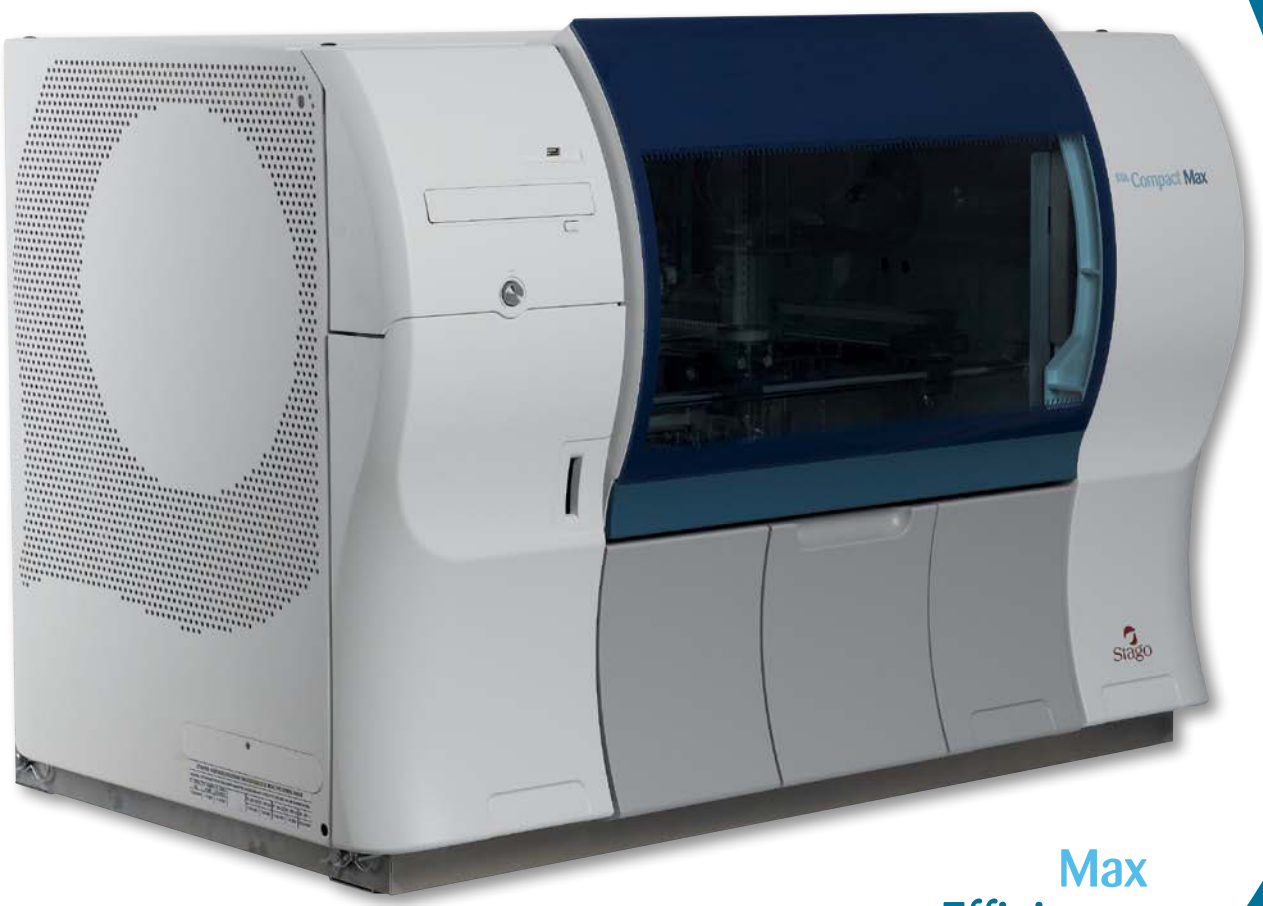

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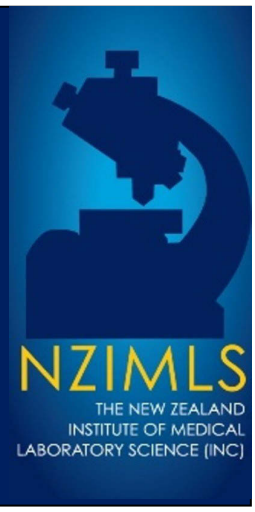

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Review article

Original articles

Case study

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Rob Siebers

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Pitfalls in the diagnosis of haematological malignancies

Rishu Agarwal and Surender Juneja

Abstract

Increased knowledge of the immunophenotypic, cytogenetic and molecular heterogeneity of haematological diseases has challenged the traditional approach to disease classification based on morphology. In the current WHO classification, an integrated approach combining clinical details, morphology, immunophenotyping and genetic features is used for the diagnosis of haematological malignancies. An accurate diagnosis is essential for proper management of patients; moreover, there are novel drugs available which are designed to specifically target the underlying pathological abnormalities responsible for the development of the tumour. However, the risk of diagnostic errors remains high in diagnostic haematology and the main reason for this is lack of resources and expertise in small centres. There is a need for access to sub-specilaised haematology services, to ensure access to specialist expertise and a full range of testing beyond traditional stains. In this review a range of potential pitfalls in the diagnosis of haematological malignancies is outlined, arising at all stages from specimen collection to reporting. Knowledge of such pitfalls, some of which are common while others are rare but of vital clinical importance, helps in avoiding potential sources of errors. Insight into the technical details has been provided which helps to avoid the errors related to sampling and processing of specimens. The final diagnosis of haematological malignancies should be based upon a combination of clinical details, good technical preparation, access to diagnostic resources, good communication and in depth knowledge and experience of the haematopathologist.

Key words: Haematological malignancies, pitfalls, diagnostic errors, centralization

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The many stages of normal haemopoeitic differentiation give rise to a number of biologically and clinically distinct cancers albeit with some overlapping features. Growing understanding of pathogenesis of haematological disorders by molecular and cytogenetic methods has significantly improved their sub-classification. However, given the overlapping features, an integrated approach combining clinical details, morphology, immunophenotyping and genetic features is now used for the diagnosis of haematological neoplasms (WHO 2008) (1). Apart from being important scientifically & biologically, an accurate diagnosis provides very useful guidance to clinicians for effective management of these patients.

Despite the advancement in technology, the rate of diagnostic errors and the requirement for expert review remains high in haematopathology. One of the main reasons for this is the lack of access to adequate range of special investigations required to make a definitive diagnosis in most centers. In 2006, the National Institute for Health and Clinical Excellence (UK) issued improving outcomes guidance (IOG) for haematological cancers, with special emphasis on the crucial need for a central regionally based specialist review of haemato-oncology diagnosis (2) and regular participation of laboratories in Quality Assurance Programmes. This component of the IOG is strongly influenced by audits, particularly the one conducted in Wales (3), showing rates of discordance of approximately 20% between initial non-specialist and subsequent specialist diagnosis of lymphomas.

In 8% of cases, the discrepancies required clinically significant changes in patient management following specialist diagnosis.

Sources of error in haematopathology can be of an administrative, clerical, technical or diagnostic nature, as in other areas of pathology. Administrative and clerical errors can occur at any stage starting from collection of specimen to the generation of final report. They are of great importance and a *regular audit system* should be in place to minimize their occurrence. Some of the most common pitfalls encountered in the daily practice of haematopathology are discussed in the six categories listed below:

- Inadequate clinical information
- Inadequate or inappropriate specimens
- Morphological diagnostic difficulties
- Errors related to immunohistochemistry
- Errors related to flow cytometry, cytogenetics/FISH and molecular studies
- Errors due to limited range of tests performed

Inadequate clinical information

Most of the haematological diseases require knowledge of clinical features—age, nodal versus extranodal presentation, specific anatomic site, and history of cytotoxic and other therapies to make the correct diagnosis. A detailed clinical history along with physical examination findings is very important to make an accurate diagnosis. Some examples where diagnostic difficulties may arise due to inadequate history and clinical details are:

- In WHO Classification, there is a subcategory of therapy related myeloid neoplasms and post transplant lymphoproliferative disorders (PTLD). Details of past treatment are essential to make this diagnosis.
- Rapidly progressive diseases like Burkitt lymphoma which is an aggressive neoplasm with rapid doubling time, requires detailed knowledge of presenting features for accurate diagnosis and timely treatment.
- While investigating for paraproteinemia, type of paraprotein should always be mentioned, as different flow cytometric immunophenotyping marker studies are required to be carried out.
- In the investigation for pancytopenia, drug history and information about organomegaly is important. For example, in rare diseases like hairy cell leukaemia, splenomegaly is a consistent clinical feature.

These are just the few examples; in reality there are very few diagnoses that can be made accurately or completely without good communication between pathologists, haematologists, oncologists and radiologists. Therefore, an integrated approach in haematology should be encouraged combining clinical details with the diagnostic modalities. **Regular clinico-pathologic meetings and well developed IT infrastructure are essential to achieve this goal.**

1. Failure to obtain adequate sample can compromise accurate diagnosis. Bone marrow aspirate and trephine biopsy should be performed by an experienced operator. The preferred anatomic site is posterior iliac crest. However, in certain conditions like the patient having received previous radiotherapy to the pelvis or there is a dry tap, sternal aspiration may be carried out. Marrow sampling from previously irradiated area can result in false negative results because the marrow may be aplastic at the irradiated site.

2. Sternal aspiration should always be performed with extreme caution because of the risk of potential damage to vital tissues including cardiac temponade associated with it. Sternal puncture should be avoided in patients with suspected plasma cell myeloma or other disorders associated with bone resorption.

3. Failure to collect samples for flow cytometry, cytogenetics and molecular studies with bone marrow aspirates can give insufficient diagnostic material. Samples for ancillary studies should be collected routinely at the time of bone marrow aspiration and may be discarded later if the investigations are considered to be unnecessary after the bone marrow slides have been reviewed.

4. Failure to make touch imprints with aspirate may prevent cytologic examination of Giemsa stained smears in cases of dry tap.

5. Obtaining an adequate length of bone marrow biopsy is very important. The length of the core from an adult should be at least 15mm. The biopsy shrinks by approximately 20% after processing. However, the larger the amount of tissue biopsied, the greater is the likelihood of a focal lesion (e.g. lymphoma, metastatic tumour, granuloma) being detected. Bilateral trephine biopsies may be performed to increase the yield of detecting focal lesions. The recommended thickness of biopsy sections is two to three microns. Thicker sections may mask subtle details of chromatin density and distribution, mitotic figures and apoptotic bodies. It is useful to examine multiple levels (three or more) of bone marrow biopsy for lymphoma staging as failure to do this may result in lower incidence of marrow involvement with lymphoma.

6. Fixation method can significantly affect morphology, cytological details and immunoreactivity. Failure to understand the use of proper fixatives can result in unsatisfactory diagnostic samples. Mercuric chloride based fixative (B5) is frequently used for bone marrow biopsies. It gives excellent cytological details but requires immediate processing and cannot be used later for molecular studies or cytogenetics. Neutral buffered formalin with ethylene-diamine tetra-acetic acid (EDTA) decalcification provides adequate morphology, preserves antigens for immunohistochemistry and nucleic acids for molecular studies. So, the choice of fixative used should be determined by the type of studies which need to be done on the specimen.

7. Decalcification of bone marrow biopsies should be carried out by reagents which allow the biopsy specimen to be used for further studies, if required. EDTA decalcification allows a wide range of immunostains with current antigen retrieval techniques. It also preserves good quality DNA for PCR studies, and FISH for lymphoma associated translocations is entirely feasible using intact EDTA- decalcified bone marrow trephine sections. Premature exposure to EDTA before adequate fixation, or exposure to poorly buffered EDTA (excessively alkaline), causes severe loss of morphological definition; it also destroys many antigens and degrades nucleic acids (5). Hence, adequate fixation and decalcification of bone marrow biopsies is very essential for accurate diagnosis.

Lymph node preparation

For the diagnosis of lymphoma, the ideal specimen is an excised intact lymph node, transported rapidly to the laboratory without previous fixation. This allows immediate sampling of the material on receipt to make imprint preparations (for rapid cytological assessment and FISH), disperse cells into medium for flow cytometry, and freeze small pieces for subsequent nucleic acid studies by polymerase chain reaction (PCR) (6). However, this ideal is rarely achievable and when fixed specimen is received, it cannot be used for flow cytometry and FISH studies. Whether received fresh or in fixative solution, lymph node specimens should be inspected and sliced as soon as possible, after recording measurements and a brief description of the intact specimen, to aid fixation.

Fine needle aspiration cytology (FNAC)

Fine needle aspiration cytology is increasingly done to minimise patient discomfort and increase the speed of diagnosis. However, FNAC only plays a limited role in screening of lymph nodes for infections, granulomas and metastatic solid tumour deposits and an excisional biopsy should be performed for the diagnosis of lymphoma.

Needle core biopsy

Needle core biopsy of inaccessible masses suspected of harbouring lymphoma is also an invaluable tool. However needle biopsies should only be used for inaccessible sites under radiological guidance and should never replace excisional biopsies because of the limited amount of tissue they provide for diagnosis.

Specimen limitations that lead to error

- Inadequate specimen size for morphology
- Inadequate sample for flow cytometry, cytogenetics or molecular studies
- Sample in wrong preservative
- Poor fixation
- Dry tap
- Crushed, necrotic or otherwise distorted tissue

Morphological diagnostic difficulties

Accurate diagnosis of haematological malignancies depends on the knowledge and expertise of the pathologist as well as availability of modern diagnostic techniques. It is very important to keep ourselves updated with the latest literature and diagnostic pitfalls. Use of ancillary techniques is advisable for definitive diagnosis. Below are some examples which cause diagnostic dilemmas on morphology:

1. Haematogones and lymphoblasts

Bone marrow haematogones often cause problems in diagnosis because of their morphological and immunophenotypic similarities to leukaemic lymphoblasts. They occur in large numbers in some healthy infants and young children and in a variety of diseases in both children and adults. Haematogones may be particularly prominent in the regeneration phase following chemotherapy and bone marrow transplantation and in patients with autoimmune and congenital cytopenias, neoplasms and acquired immunodeficiency syndromes. In some instances they constitute 5% to more than 50% of all nucleated cells (7). They pose important diagnostic challenge when evaluating acute lymphoblastic leukemia (ALL) bone marrow post chemo therapy marrow. They are often expanded in regenerating marrow and can potentially be mistaken for residual disease. While morphologic and immunophenotypic overlap exists between haematogones and leukemic lymphoblasts, a careful morphological review combined with evaluation of immunophenotypic characterstics by flow cytometry and immunohistochemistry as well as architectural distribution in the bone marrow can help distinguish haematogones from residual leukemic blasts.

Haematogones show continuous and complete maturation spectrum on immunophenotyping, lack aberrant or asynchronus antigen expression while lymphoblasts from ALL deviate from the normal B-lineage maturation spectrum by exhibiting maturation arrest, over or under antigenic expression, asynchronus antigen expression or expression of myeloid associated antigens.

2. Metastatic mimickers of acute leukemias

Metastatic involvement of the bone marrow by malignant small round cell tumour is not uncommonly encountered in paediatric patients, and these tumours include neuroblasoma, Ewings sarcoma and rhabdomyosarcoma. In some patients, bone marrow disease may be the primary manifestation, thus mimicking acute lymphoblastic leukemia. Cases of rhabdomyosarcoma mimicking acute leukemia have been described in the literature (8). Similarly, a case of metastatic small cell carcinoma of the lung has been reported mimicking acute leukemia (9). We should therefore be vigilant while making a diagnosis of acute leukemia especially in those cases which on immunophenotyping show abnormal cells neither to be of B or T cell origin and do not show reactivity for myeloid antigens. In addition to considering the diagnosis of acute undifferentiated leukemias, possibility of metastatic tumours should always be kept in mind. A detailed work-up of such cases including detailed history and radiological findings, bone marrow biopsy examination, extended panel of antibodies by immunohistochemistry and relevant cytogenetic analysis should always be carried out to make a correct diagnosis.

3. Hypocellular AML

Rare cases of AML present with moderate to markedly hypocellular marrow. Causes of this reduced cellularity are unknown. The key challenge in these cases is to document that blasts exceed the 20% threshold. Distinction from hypocellular myelodysplastic syndrome (MDS), hypocellular hairy cell leukemia and aplastic anemia can usually be achieved by integration of morphology, immunohistochemistry, immunophenotyping, cytogenetics and molecular studies.

4. AML with fibrosis

Fibrosis is highly characteristic of acute megakaryoblastic leukemia and therapy related myeloid neoplasms. Because of the difficulty in obtaining bone marrow aspirate in these fibrotic bone marrows, definitive diagnosis of AML often relies upon immunohistochemical assessment of bone marrow biopsy sections to estimate the number of blasts and myeloid expression by them.

5. Reactive vs neoplastic conditions:

Misdiagnosis of reactive lesion as neoplastic or vice versa can be obviously deterimental for the patients as well as have medicolegal implications for the pathologist. Therefore, all effort should be made by the diagnostic team to avoid these errors. Some of the common benign conditions which mimic malignancy are:

- Kikuchi lymphadenitis**-** a self limited disease of young women, frequently misdiagnosed as lymphoma due to many histiocytes and many activated T cells in lymph nodes.
- Infectious mononucleosis and other viral infections, reaction to vaccines and drug hypersensitivity, often cause diagnostic confusion due to reactive immunoblastic proliferation and large activated lymphoid cells. Reed-Sternberg like cells can also be seen which causes one to consider differential diagnosis of Hodgkin lymphoma.
- Castleman's disease- especially plasma cell variant which in small biopsies can be misdiagnosed as an extraskeletal plasmacytoma or marginal zone lymphoma.
- Autoimmune lymphoproliferative syndromes: This is due to non functional or dysfunctional apoptosis of lymphocytes. Because of impaired apoptosis, lymphoid cells that have proliferated in response to antigens or infections persist after the challenge is over, causing persistent enlargement of the reticulo-endothelial system, manifesting as generalised lymphadenopathy and hepatosplenomegaly. This often causes resemblance to peripheral T cell lymphoma (PTCL); however, diagnosis of PTCL should be made with caution in young children and after thorough investigations.

6. Low grade vs high grade lymphoma

Different types of lymphomas tend to have very varied clinical course including survival rates and therefore an accurate diagnosis is critical. Failure to appreciate the higher biological grade of some lymphomas composed of small cells can be a cause of misdiagnosis. Mantle cell lymphoma, lymphoblastic lymphoma and even Burkitt lymphoma can fall into this category. In thick sections, intense nuclear staining may mask subtle details of chromatin density and distribution that normally assist in the distinction between small lymphocytes, lymphoblastic and Burkitt type cells. It may also make mitotic and apoptotic bodies difficult to recognise against the tissue background. Immunostaining and other diagnostic investigations like MYC translocations for Burkitt lymphoma should always be carried out.

7. Hodgkin lymphoma vs non Hodgkin lymphoma

It is important to distinguish Hodgkin lymphoma (HL) from non-Hodgkin lymphoma (NHL) because of different treatments and clinical outcomes. Histological criteria for diagnosing HL are well defined and few years ago it was considered so straightforward that no immunostaining or specialist opinion was considered important. However, the distinction between HL & NHL can be difficult in some cases; in fact, a specific entity *B cell lymphoma, unclassifiable, with features intermediate between DLBCL and HL* is defined in the 2008 WHO classification. These lymphomas generally have a more aggressive clinical course and poorer outcome than classical Hodgkin lymphoma or diffuse large B cell lymphomas. Hence, when no regular immunostaining is used in HL, there could be a potential problem of failure to recognize atypical cases that might instead be NHL. Reed-Sternberg cells are considered as diagnostic hallmark for HL. However, they can be found in many reactive conditions like infectious mononucleosis, after drug therapy and some NHL.

8. DLBCL vs Burkitt lymphoma

BL is a highly aggressive neoplasm with extremely short doubling time and correct identification is important due to the need for higher intensity treatment as compared with other lymphomas. Misdiagnosis as DLBCL may lead to under-treatment of patients who might otherwise be cured. There can be considerable overlap between BL and DLBCL and morphology alone should never be the basis for distinguishing these two entities. Immunophenotyping is essential and can be very helpful (the neoplastic B cells of BL are typically CD10 positive and BCL2 negative) but variant immunophenotype can be seen in BL. Ki-67 is another helpful marker and in BL it is close to 100%. However, some DLBCL also approach this value and their true biological nature is being questioned. Demonstration of c-MYC translocation by FISH is considered as gold standard for the diagnosis of BL. However, this is complicated by the occurrence of MYC abnormalities in some DLBCL. Cases of DLBCL with MYC abnormalities are invariably aggressive and require higher intensity treatment in comparison to other DLBCL. To fit these grey zone cases, *category of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL* has been added in WHO classification.

9. Other important grey areas

Some lymphoproliferative disorders like CD23 negative chronic lymphocytic leukemia's, CD5 negative mantle cell lymphomas, CD5 positive follicular lymphomas, CD10 negative follicular lymphomas and CD5 positive splenic marginal zone lymphomas also exist and should always be considered in differential diagnosis in appropriate cases.

10. Post Rituximab changes

Treatment with Rituximab often causes downregulation of CD20 expression. So, in a patient previously treated with Rituximab, CD20 antibody should not be used for B lineage assessment. Instead, other markers like PAX, CD79a, OCT-2 & BOB.1, should be used. PAX5 is a nuclear stain which gives excellent staining and is very helpful in identifying B cells in Rituximab treated cases.

Errors related to immunohistochemistry (IHC)

Immunohistochemistry is an essential component in the diagnosis of haematological neoplasms. We should therefore be familiar with technical and interpretive pitfalls, since many factors may influence the technical preparation of the immunoreactions and a wide variety of causes can result in incorrect interpretations.

Fixatives

Length and type of fixation may influence immunohistochemical staining. Specimens for immunohistochemistry should be fixed immediately as drying can result in non-specific staining. 10% neutral buffered formalin is the most commonly used fixative for histology specimens. Mercury based fixatives such as B5 and Zenkers are also used in bone marrow trephine biopsies as they provide excellent nuclear details. However, they induce molecular cross linking and may hamper reactivity of a number of important antigens, for example CD4, CD5, CD10, CD23 and sometimes CD30 (10-12). On the contrary, kappa and lambda light chain staining appears better in B5 than in formalin fixed tissues (10). The length of fixation also affects the results of immunostaining as underfixation often produces a reduced immunostaining in the central region of the tissue block with stronger immunoreaction in the marginal area of the section while overfixation generates the opposite effect (13).

Decalcification

Decalcification by strong acid on bone and bone marrow samples can have a negative effect on detection of CD markers. Good quality immunohistochemistry has been reported using formalin fixation with ethylene-diamine tetra-acetic acid (EDTA) decalcification.

Antigen retrieval

Different methods for antigen retrieval are used such as enzyme or protease induced antigen retrieval or heat induced antigen retrieval. Successful retrieval depends upon factors like fixation time, temperature, pH and molarity of the solution. A standard protocol should be developed by individual laboratories and should be strictly followed.

Inappropiate controls

For the validation of IHC results, relevant positive and negative controls should always be run. Maintaining supplies of relevant positive control tissue can be problematic, but is essential. For bone marrow biopsy, as far as possible, controls should be applied on specimens which are fixed and decalcified in the same way as the tissue for diagnosis.

Knowledge of normal expression

It is also essential to know what internal control reactivities to expect in normal/ reactive as well as neoplastic lymphoid tissues. Internal controls are elements of diagnostic tissue itself that help provide reassurance that immunostaining has worked. Normal reactivities should not be misinterpreted as pathological, for example, endothelial cell expression of CD34 and expression of cyclin D1 by occasional bone marrow stromal cells.

It is crucial to know the normal distribution of antigens and their anticipated variation in neoplasia. A key example is bcl2 expression in follicular lymphoma. There is no bcl2 expression in reactive germinal center B cells but as a result of t (14; 18) they are expressed in germinal center B cells. However, bcl2 expression is normal in T lymphocytes which can sometimes be prominent in germinal centers and peripheral B cells. So the interpretation of bcl2 expression should be made with caution by an experienced observer. In summary, in IHC, every brown cell is not positive and positivity should be interpreted with the detailed knowledge of antibody expression in normal and malignant cells.

Errors related to flow cytometry

Flow cytometry is a very useful tool in diagnostic haematology. The introduction of multicolour cytometry has enabled the rapid diagnosis of haematological neoplasms to be routinely achieved. The advantages of flow cytometry are rapid turnaround time, small specimen volume, identification of multiple antigens simultaneously, demonstration of monoclonality and detection of weakly expressed antigens. However, flow cytometry requires a *high degree of expertise and a thorough understanding of technical details* for a meaningful analysis. Some of the important issues which one should address before interpreting flow cytometry data are:

- Characterstics of flourochromes: The flourochromes chosen should be biologically inert i.e. they should not bind normal cells resulting in background staining, they should be stable and readily conjugate to the monoclonal antibody of choice without inducing conformational changes to the surface antigens through mechanisms such as charge interactions. Ideally they should also produce a bright fluorescent signal resulting in a high signal to noise ratio.
- Compensation: Compensation is the process by which the spectral overlap between different fluorochromes is mathematically eliminated. Compensation adjustments are routinely applied in all flow cytometry experiments, but in many instances the proper application of this vital step is poorly understood. In order to properly analyse experimental data, users must be aware of the effects of compensation, understand how to apply it correctly and recognize when data are not properly compensated.
- Choosing the right flourochrome: As mentioned above, fluorochromes differ in the relative brightness of the signal they produce. Conjugation of various fluorochromes to the same antibody, and staining of the same population of cells, can therefore result in large differences in resolution of positive and negative events. This difference may be largely inconsequential for highly expressed markers but is of great importance for surface markers that exhibit low levels of expression. The general rule of thumb therefore is that the lower the level of antigen expression, for a given marker, the more important it is to use a bright fluorochrome in order to generate superior results.
- Proper use of isotype controls: Isotype controls have historically been used to assess the background staining level and set criteria to differentiate between positive and negative expression of antigens. This can be problematic when the isotype control does not precisely match the analytical antibody in terms of antibody class, protein concentration and fluorescein to protein ratio. Thus, over reliance on isotype controls carries a risk of misinterpretation and in many instances the use of “internal controls” provides a more robust understanding of the antigen expression of a given cell population.
- Use of appropriate gating strategies: In flow cytometric analysis of a heterogeneous population of cells it is usual to apply electronic “gating” so that only the subset of cells of interest is displayed for interpretation. An example is the use of a CD45 vs side scatter gating strategy in the diagnosis of leukemias and lymphomas to delineate the population of interest. There are several alternative approaches including forward scatter vs side scatter that may be used to differentiate cells of different lineages, and “lineage” gating – where a lineage specific monoclonal antibody is used to select cells of interest. Irrespective of the strategy used, if the cellular population of interest is excluded from the gate, then erroneous results will be obtained.

This is particularly true when a "live" gating approach is employed such that fluorescent data from ungated events is discarded making it impossible to perform retrospective regating of data files.

- Adequate sample: As with all diagnostic modalities, an adequate and representative sample is necessary for meaningful analysis. Sometimes due to a sclerotic or markedly hypercellular marrow, sufficient sample cannot be obtained for analysis. Careful correlation with clinical history and other diagnostic details is necessary in such situations to ensure accurate diagnosis.

Cytogenetics/FISH studies

Cytogenetics and FISH studies play an important role in the diagnosis of haematological neoplasms. They also help in deciding management of patients as well as to predict prognosis, response to treatment and disease progression. An example is demonstration of MYC translocation for the diagnosis of Burkitt lymphoma.

Conventional cytogenetics are helpful in identifying chromosomal abnormalities but are limited due to the poor quality of metaphases and low mitotic index associated with many diseases. As a result, a significant proportion of bone marrow karyotypes are reported as normal by cytogenetic studies. FISH helps to overcome this problem by using non dividing cells as target (interphase FISH), and allowing for the identification of both numerical and structural abnormalities in a large number of cells. This has considerable advantage for some haemopoietic malignancies, where the proliferative activity is low, or when dividing cells do not represent the neoplastic clone. For example, in the cytogenetic studies of B-CLL with interphase FISH, a much higher incidence of trisomy 12 is found as compared to conventional cytogenetics. However, FISH is a more targeted approach and generally requires prior knowledge of anomaly of interest, so cannot be used as a screening tool. Also, the number of commercially available probes is limited and interpretation can be challenging with FISH when analysing suboptimal sampling (especially background fluorescence with formalin fixed paraffin embedded tissue). Hence, one should understand the limitations of these techniques and also should keep in mind that they are adjuncts and should not form the sole basis for a diagnosis.

Conventional cytogenetics requires fresh tissue, so the lymph nodes tissue should be sent to the laboratory fresh before formalin fixation. For blood and bone marrow, samples should be collected in heparin as per laboratory guidelines and sent immediately to cytogenetic laboratory. FISH can be performed on fresh, frozen or paraffin embedded tissue and can provide results when there is insufficient tissue for conventional cytogenetics.

It should also be remembered that cytogenetics and FISH studies cannot be performed on tissue fixed in mercury based fixatives (eg Bouin's solution for bone marrow biopsies), so sample should be collected in appropriate medium for these studies.

Molecular haematology

Molecular techniques can contribute to establishing the correct diagnosis, prognostic stratification and predicting and assessing response to treatment in haematological malignancy. For example, presence of JAK2 mutations confirms a suspected diagnosis of a myeloproliferative neoplasm, PML-RARA confirms acute promyelocytic leukemia (APML), and BCR-ABL1 confirms chronic myeloid leukemia (CML) and FIP1L1-PDGFRA confirms chronic eosinophilic leukemia. Examples of targeted therapies based on molecular markers are all-transretinoic acid in APML and imatinib in CML. Success of molecular haematology analysis largely depends upon tissue type, sample quality and sensitivity of assay used. The sample required is usually peripheral blood or bone marrow in EDTA which should be sent to molecular laboratory as soon as possible.

A major problem in diagnosing neoplasia by molecular methods is the background of normal cells which are invariably present in tumour specimen. For example, in systemic mastocytosis, the KIT mutation (D816V) may not be reliably detected if there are insufficient numbers of mast cells (14). Enrichment techniques are usually applied which improves sensitivity of the techniques.

Contamination is also a major problem with molecular techniques and can result in false positive results. Care should be taken to minimize contamination, including setting up reactions in a dedicated PCR cabinet, keeping pre-PCR and post-PCR areas separate, decontaminating equipment regularly and rigidly adopting good laboratory practice at all times.

In some cases, when the level of positivity is low as in MRD or some rare genetic abnormality is identified, it is always advisable to run the test in duplicate and correlate with other test results.

False negative results are often due to poor sample quality. Tests based on RNA analysis are highly sensitive because RNA is labile and prone to rapid degradation by RNAase enzymes which are abundant in the environment. Samples for RNA analysis must therefore be processed within 48-72 hrs. Sometimes, false negative results also occur if wrong molecular target is monitored. This may occur if fusion break points at diagnosis are not established, and an assumption made that the patient has a common fusion type that can be detected with standard primers. One should keep these limitations in mind and should have adequate experience in molecular techniques in order to get accurate results.

Errors arising due to limited range of tests done

Haemotoxylin and eosin staining alone is insufficient in making the diagnosis in many cases of haematopathology and an extended panel of antibodies as well as genetic tests is required to reach accurate final diagnosis. Sometimes, due to limitations in the availability of tests, only limited investigations are performed which may lead to inaccurate diagnosis. For example, leaving a diffuse small lymphoid cell infiltrate categorised as small B cell lymphoma without investigating CD5, CD23, cyclin D1, and Ki-67 expression to differentiate chronic lymphocytic leukemia/ small lymphocytic lymphoma, mantle cell lymphoma or marginal zone lymphoma is incorrect as these diseases are often treated with different protocols. Mantle cell lymphoma is an aggressive disease as compared to chronic lymphocytic leukemia, although both are small lymphocytic proliferations.

Along with the diagnosis, there is an increasing need to evaluate prognostic markers like CD10, BCL2, BCL6 and MUM1 to subcategorize DLBCL into Germinal Centre (GC) type or Activated B Cell (ABC) type as they have different survival rates.

Several additional algorithms involving antibody combinations based on gene expression profiling have been proposed for improved prognostication in aggressive B cell lymphomas. It is also understandable that all these diagnostic modalities are not available in every centre, *so a centralized referral haematology department should ideally be established in each region which is well equipped with modern diagnostic facilities and experienced staff and it should serve as the referral centre for all cases*.

Conclusion

The final diagnosis in haematological malignancies should be based upon a combination of the following:

- Clinical details: Adequate history as well as results of additional tests (in particular haematological/ biochemical investigations and imaging).

- Technical preparation: Sufficient sample in proper preservative analysed by experienced scientific officer/ pathologist.
- Resources: Facilities for immunohistochemistry, flow cytometry and genetic studies.
- Knowledge: Familiarity with WHO Classification, access to guidelines and references, experience in interpretation of flow cytometry and genetic results.
- Experience: In differentiating reactive or benign and malignant conditions as well as proper application of ancillary techniques in diagnosing them. And finally the ability to refer difficult cases to a centralized laboratory which is well equipped and well staffed by experts.
- Deficiency of one or more of the above has the potential to compromise accurate diagnosis of haematological malignancies.

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References

1. Harris NL, Campo E, Jaffe ES, Pileri SA, Stein H, Swerdlow SH, Thiele J, Vardiman JW. Introduction to the WHO classification of tumours of haemopoeitic and lymphoid tissues. In: SH Swerdlow, E Campo, NL Harris, ES Jaffe, SA Pileri, H Stein et al, eds. WHO Classification of Tumours of Haemopoeitic and Lymphoid Tissues, Fourth Edition. IARC, Lyon, France, 2008: 14-15.
2. National Institute for Health and Clinical Excellence. http://guidance.nice.org.uk/ CSGHO (accessed 7 Feb 2011).
3. Lester JF, Dojcinov SD, Attanoos RL, O'Brien CJ, Maughlan TS, Toy ET et al. The clinical impact of expert pathological review on lymphoma management: a regional experience. *Br J Haematol* 2003; 123: 463-468.
4. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC. ICSH guidelines for the standardization of bone marrow specimens and reports. *Int J Lab Hematol* 2008; 30: 349-364.
5. Wilkins BS. Pitfalls in bone marrow pathology: avoiding errors in bone marrow trephine biopsy diagnosis. *J Clin Pathol* 2011; 64: 380-386.
6. Wilkins BS. Pitfalls in lymphoma pathology: avoiding errors in diagnosis of lymphoid tissues. *J Clin Pathol* 2011; 64: 466-476.
7. McKenna RW, Washington LT, Aquino DB, Picker LJ, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry. *Blood* 2001; 98: 2498-2507.
8. Shinkoda Y, Nagatoshi Y, Fukano R, Nishiyama K, Okamura J. Rhabdomyosarcoma masquerading as acute leukemia. *Pediatr Blood Cancer* 2009; 52: 286-287.
9. Taetle R, Wohl H. Oat cell carcinoma mimicking acute leukemia. *West J Med* 1978; 129: 497-500.
10. Arnold MM, Srivastava S, Fredenburgh J, Stockard CR, Myers RB, Grizzle WE. Effects of fixation and tissue processing on immunohistochemical demonstration of specific antigens. *Biotech Histochem* 1996; 71: 224-230.
11. Dorfman DM, Shahsafaei A. Usefulness of a new CD5 antibody for the diagnosis of T-cell and B-cell lymphoproliferative disorders in paraffin sections. *Mod Pathol* 1997; 10: 859-863.
12. Facchetti F, Alebardi O, Vermi W. Omit iodine and CD30 will shine: a simple technical procedure to demonstrate the CD30 antigen on B5 fixed material. *Am J Surg Pathol* 2000; 24: 320-322.
13. Leong AS, Gilham PN. The effects of progressive formaldehyde fixation on the preservation of tissue antigens. *Pathology* 1989; 21: 266-268.
14. Garcia Montero AC, Jara-Acevedo M, Teodosio C, Sanchez ML, Nunez R, Prados A, et al. KIT mutation in mast cells and other bone marrow haemopoeitic cell lineages in systemic mast cell disorders: a prospective study of Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood* 2006; 108: 2366-2372.

Comparison of two methods for the diagnosis of chronic granulomatous disease - neutrophil oxidative burst measured by the nitroblue tetrazolium slide test versus the dihydrorhodamine 123 flow cytometric assay

Geri Dimitrova, Carolyn Bunkall, Danny Lim and Christopher Kendrick

Abstract

Objective: The neutrophil respiratory burst is crucial for the ability of the host to kill ingested microorganisms. The detection of this activity is an essential part of the laboratory investigation of patients with suspected chronic granulomatous disease (CGD). In this study the traditional qualitative nitroblue tetrazolium (NBT) slide test was compared with a quantitative whole blood dihydrorhodamine 123 (DHR 123) assay using flow cytometry.

Methods: A total of 20 samples submitted to Labplus at Auckland Hospital were screened for CGD by both the NBT and the DHR 123 assays.

Results: While the NBT method was able to demonstrate reduced NADPH oxidase in CGD patients, it is highly subjective and cannot identify carrier states of X-linked-type CGD. In contrast, the quantitative whole blood dihydrorhodamine 123 (DHR 123) flow cytometric method evaluated in this study was more rapid, allowing the proportion of affected cells to be determined, and was able to identify the carrier state of X-linked CGD.

Conclusions: The DHR 123 assay proved to be a more sensitive and more convenient method for the measurement of neutrophil oxidative burst activity and showed a number of advantages over the qualitative NBT slide test for the diagnosis of CGD.

Key words: chronic granulomatous disease, nitroblue tetrazolium slide test, dihydrorhodamine 123, flow cytometry, neutrophil respiratory burst

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Introduction

Polymorphonuclear neutrophils (PMNs) destroy microorganisms by producing reactive oxygen species (ROS) during the respiratory burst, a normal host defence mechanism that controls infections. The process is controlled by the multicomponent enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, with the production of reactive oxygen species with the formation of superoxide anion (O_2^-) (1). Intracellularly the O_2^- is converted by superoxide dismutase (SOD) to oxygen and hydrogen peroxide (H_2O_2), with the latter converted by myeloperoxidase (MPO) to hypochlorous acid and chloramines inside the phagolysosome. These toxic products of the oxidative burst are a part of the powerful O_2-dependent antimicrobial system of PMNs (1, 2).

The clinical implication for impaired function of cellular NADPH oxidase, can be seen in patients with chronic granulomatous disease (CGD) (1).

In this genetic disorder, neutrophils and monocytes recognise and ingest, but are not able to kill certain microorganisms, because phagocytes fail to generate O_2^-. This rare condition is associated with recurrent bacterial and fungal infections and as a result patients develop serious, often life-threatening, and unusually persistent infections (1). CGD patients most commonly present with upper respiratory tract infections, pneumonia and abscesses of the skin, tissue and other organs, arthritis, osteomyelitis, cellulitis, and impetigo. Granulomas form due to the accumulation of white blood cells in the infected areas, even in cases when antibiotics have eliminated the infection-causing organism.

Patients with CGD are especially susceptible to infections caused by catalase positive organisms such as *S. aureus, E. coli* and *Aspergillus* species as catalase produced by these organisms destroys any endogenously generated H_2O_2. In contrast, the neutrophils of CGD patients are usually able to destroy catalase negative organisms such as pneumococci, streptococci and others because of the H_2O_2 formed against the organism within the phagolysosome. CGD can be inherited as either an X-linked or autosomal recessive disorder (Figure 1) with the genetic lesion caused by deletion, frame-shift, nonsense or missense mutations in the gene or gene promoters (3).

Most clinical cases of CGD present in childhood and early diagnosis is crucial as it may require patients to be placed on antibiotic prophylaxis in an attempt to protect against infections before they occur. Since NADPH is a cofactor for the production of superoxide dismutase, other conditions which affect the formation of NADPH can also lead to impaired neutrophil function. This can sometimes also be seen in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiencies (4).

The detection of the defect in the neutrophil respiratory burst activity is an important part of the investigation of patients with CGD (5). The NBT slide test has been the traditional laboratory test for the diagnosis of CGD and has been offered for many years at LabPlus. In the NBT test, the blue dye formazan is produced by the reduction of the NBT dye, a reaction dependent upon neutrophil NADPH oxidase (2). The NBT test is dependent upon the microscopic evaluation of stained neutrophils for the presence of intracellular formazan. The NBT method was replaced recently by the dihydrorhodamine 123 (DHR) assay, a flow cytometry method that uses an anticoagulated whole blood specimen. DHR 123 is a fluorogenic substrate for the respiratory burst and it is oxidised to rhodamine by cellular hydrogen peroxide. Intracellular rhodamine formation is fluorescent and is detected and measured using a flow cytometer. In this study, the NBT slide test and the whole blood DHR assay were compared.

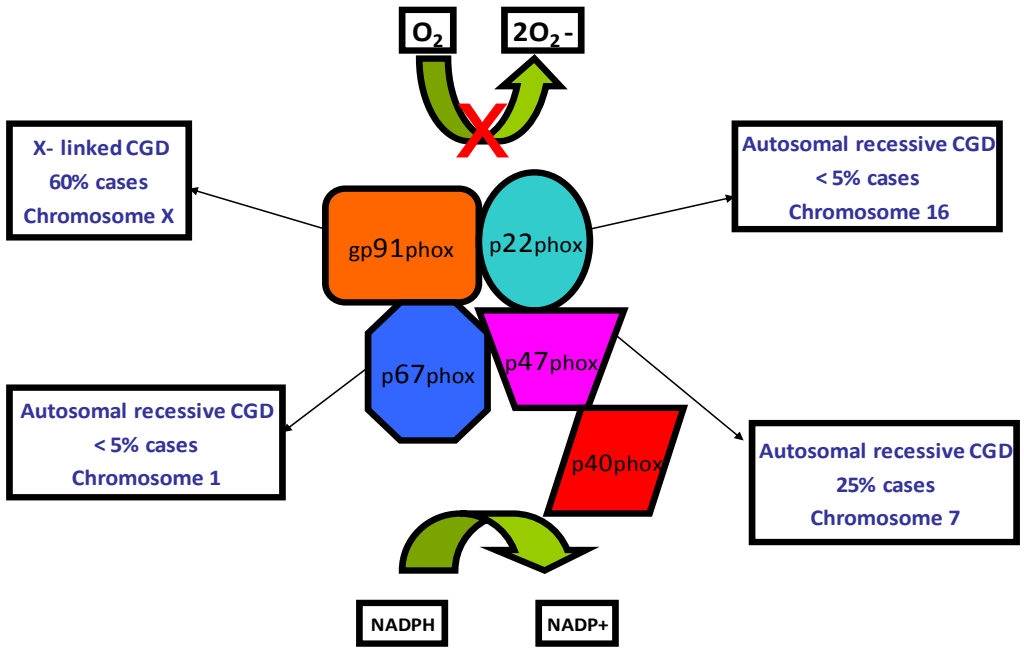


Figure 1. Mode of inheritance, defective subunits of NADPH oxidase and chromosomal assignments that can lead to CGD.

Materials and methods

All patient and control samples for the NBT assay were collected by laboratory staff by finger-prick. Blood was collected into plain capillary tubes at the patient's bedside. Samples used for the DHR assay had been previously collected by venesection into EDTA anticoagulant.

Nitroblue tetrazolium test (NBT)

The NBT test involves the microscopic assessment of the ability of patient neutrophils to reduce the yellow soluble redox dye NBT to form blue-black formazan, an insoluble material that precipitates intracellularly. The degree of production of formazan provides a qualitative means of identifying the presence of neutrophil superoxide anion O_2^- following stimulation.

Complement was used as opsonin for the NBT test and was provided using fresh pooled human serum. A NBT solution of 0.28% in normal saline and a zymosan (yeast) solution of 1% in normal saline was used. The glass adherence reagent consisted of 0.5mL of fresh human serum, 0.6mL NBT solution and 0.3mL normal saline. The zymosan reagent was prepared using 0.5mL of fresh human serum, 0.6mL of NBT solution and 0.3mL zymosan solution.

Two clean glass slides were labelled "glass" and "zymosan" for both the "normal" and "patient" samples and two circles drawn on each slide and whole blood from both the patient and a normal control were applied to the slides. Slides were placed horizontally in a petri dish with moistened filter paper and incubated at 37˚C for 25 minutes. Any blood clots were gently washed off the slides with normal saline, care being taken to avoid scraping of the cell layer that could disturb the adherent neutrophil monolayer. To the areas of the slides labelled "glass", glass adherence reagent was added. To the "zymosan" area of the slides a few drops of zymosan reagent was added. Slides were again placed in a humidity incubator at 37˚C for 20 minutes and rinsed with normal saline and allowed to dry.

Slides were fixed in methanol for 2 minutes and stained with 0.1% neutral red for 10 minutes. Excess stain was rinsed off with water and slides left to dry.

Adequacy of the counter stain of the adherent neutrophils was checked under the microscope prior to being lacquered. Slides were examined microscopically and the number of adherent PMN cells showing NBT reduction among 100 consecutive PMNs was recorded as a percentage.

Dihydrorhodamine flow cytometry assay

Neutrophils are first stimulated using phorbol 12-myristate 13-acetate (PMA). This initiates intracellular O_2^- production and dihydrorhodamine 123 (DHR) is oxidised by the neutrophil reactive oxygen species (ROS) to rhodamine 123 (RHO) releasing a fluorescent green signal which can be measured by flow cytometry. Since RHO also binds to cellular membranes, the fluorescent signal is exclusively contained within the cells (1). Normal bloods produce a strong fluorescence whereas patients with an abnormality in O_2^- production produce a weak level or no fluorescence at all. Carriers of the X-linked CGD gene show the presence of two peaks that represent the presence of a single defective CGD gene and a functionally normal gene. In these cases the results show a strong positive peak and a separate weaker/ negative peak. DHR is reportedly the most effective flow cytometric probe for assessing the oxidative burst in human granulocytes and is specifically responsive to H_2O_2 accumulation (1).

In the method stock DHR 123 (Sigma) was dissolved in phosphate buffered saline (PBS) to give a working solution of 30µg/mL. CD45 antibody (Becton Dickinson) was conjugated with peridinin-chlorophyll-protein complex (CD45 PerCP). Phorbol 12-myristate 13-acetate (PMA) (Sigma) was dissolved in dimethyl sulphoxide (DMSO) (Sigma) to form a 1.6mM solution. Stock FACS lyse solution (Becton Dickinson) was diluted 1 in 10 in deionised water to give a working solution.

Residual EDTA anticoagulated whole blood was used and all specimens were tested within 24 hours from the time of collection. Samples requiring testing the next day were stored at 2-8˚C overnight. Phorbol 12-myristate 13-acetate (PMA) was used to activate neutrophils in the assay. Three *fluorescence activated cell sorting* (*FACS*) tubes were labelled as "blank", "resting" and "stimulated" for patient and control samples. 10µL of CD45 antibody conjugated with peridinin-chlorophyll-protein complex (CD45-PerCP) was added to each tube. 50uL of mixed patient and control bloods were added to each of the three patient and control FACS tubes. 12.5µL of PBS was added to the "blank" and "resting" tubes and 12.5µL of working PMA solution added to FACS tubes labelled "stimulated" and incubated at 37˚C for 15 min. 12.5µL of PBS was then added to tubes labelled "blank" and 12.5µL of working DHR solution to the "resting" and "stimulated" sample tubes. After 5 minutes at 37˚C, 1.0 mL of FACS lyse solution was added to all tubes. Tubes were left in the dark at room temperature for 15 min and the cells washed twice with 2ml of PBS. Samples were immediately analysed using the Becton Dickinson FACSCANTO II (San Jose, CA, USA) flow cytometer.

Results

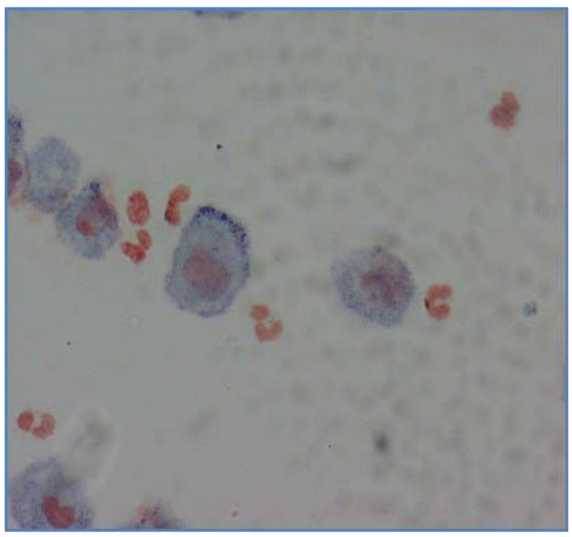
NBT test:

For the adherence slides labelled with "glass", the NBT reduction was seen as a blue to purple/black formazan staining surrounding the polymorphs (Figure 2A). For the "zymosan" stimulated slides, NBT reduction was seen as a blue to purple/ black precipitate in the polymorphs that had ingested the yeast and reduced the NBT (Figure 2B).

The NBT test took an average of 3.5 hours to perform and sixteen tests showed results within the normal range values (>30% glass adherent cells and >90% zymosan stimulated cells). Four test results fell outside of the normal range values for the dye reduction in the "glass" adherent and "zymosan" stimulated neutrophil groups.

These four samples showed either no reduction of the NBT dye or the percentage of cells that reduced the dye was lower than the normal range, producing glass adherent neutrophils with formazan deposits in fewer than 30% (Figure 3A) and the lack of formazan production (Figure 3B).

A



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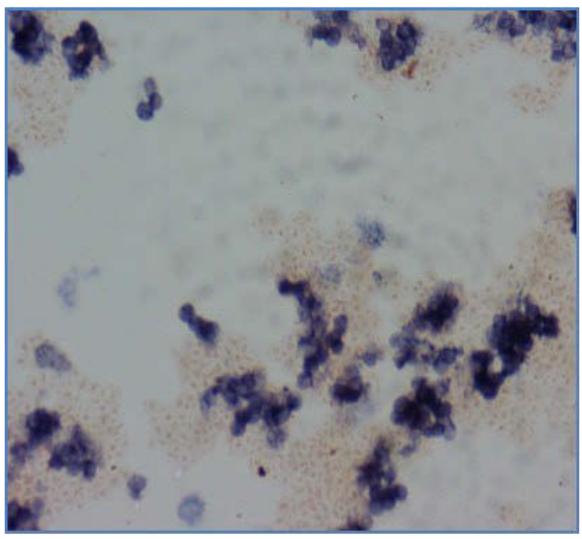
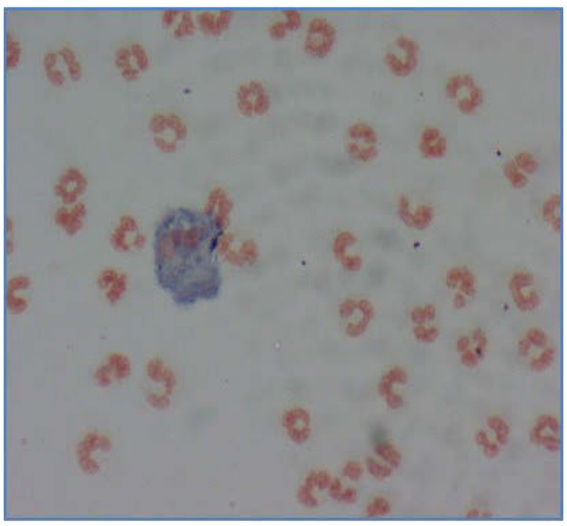


Figures 2A & 2B. Nitroblue tetrazolium blue formazan staining of neutrophils (A) and the blue to purple black precipitate in the zymosan stimulated neutrophils (B) in a normal patient.

A



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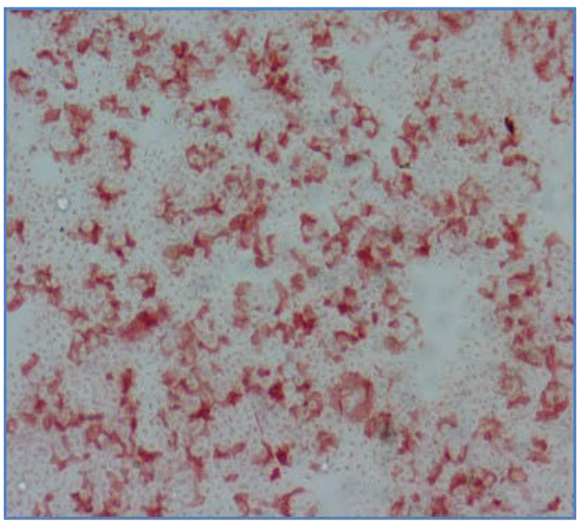

Figures 3A & 3B. Reduced or no formazan staining of neutrophils (A) and no blue/black deposit staining in zymosan stimulated neutrophils(B) in patients with CGD.

DHR assay

Data was collected from the reagent “blanks”, “resting” and “stimulated” FACS tubes with the flow cytometer recording 10,000 neutrophil events. At analysis the scattergram of CD45 expression vs. side scatter was first displayed and the neutrophil population was identified by its typical location and selected by gating (Fig. 4). Gating of CD45 positive cells in this region selects for neutrophils and excludes debris and other cells present in the samples. A histogram of rhodamine fluorescence was obtained for the cells in the gated region of the flow scatterplot (Figures 5-7) for each of the samples.

At analysis the neutrophil populations of both patient and control samples was readily identifiable on the CD45 scattergram (Figure 4). The “blank” samples showed the lowest rhodamine fluorescence (Figure 5A, 6A, and 7A), with “resting” neutrophils showing a slight increase in fluorescence uptake (Figure 5B, 6B, 7B). PMA stimulated neutrophils produced a large increase in rhodamine fluorescence in samples from patients with normal NADPH oxidase activity (Figure 5C). The same level of fluorescence was not seen in samples from patients with X-linked CGD (Figure 6C). Two separate fluorescence patterns were observed in two samples (Figure 7C) from patients known to be X-linked CGD carriers.

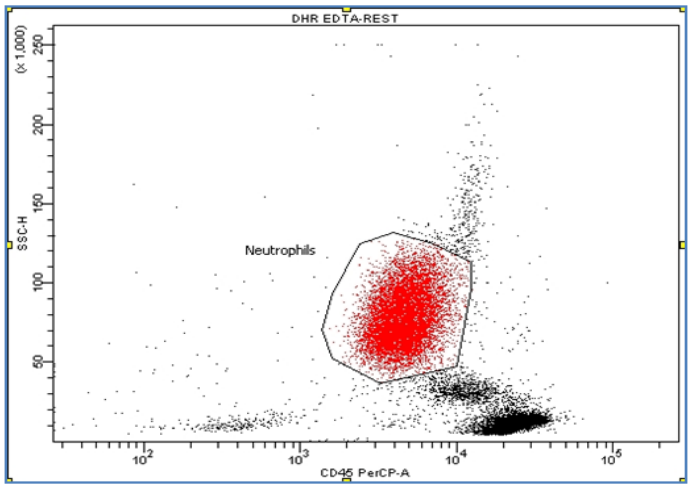

Figure 4. A flow cytometry dot plot of CD45 vs. side scatter of a whole blood specimen. White cells are differentiated by their characteristic CD45 expression - intensity staining on the x axis, and granularity - side scatter on the y axis. In this “resting” sample, the neutrophil population has been gated.

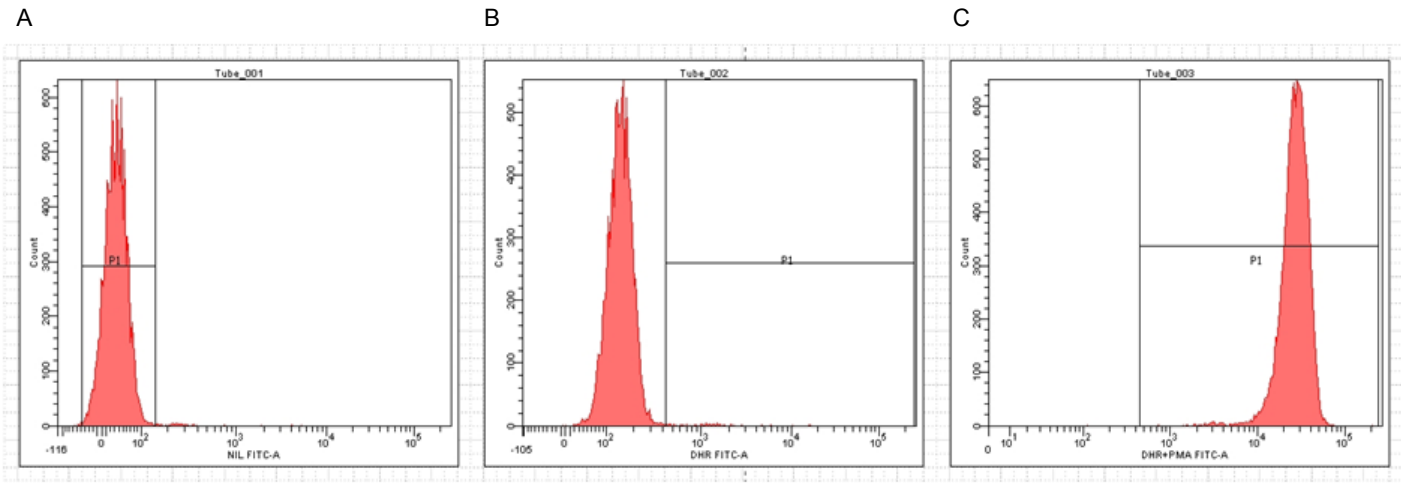

Figure 5. Flow cytometry histograms showing DHR rhodamine fluorescence for normal neutrophils. (A) “blank”, (B) “resting” neutrophils, and (C) “stimulated” neutrophils.

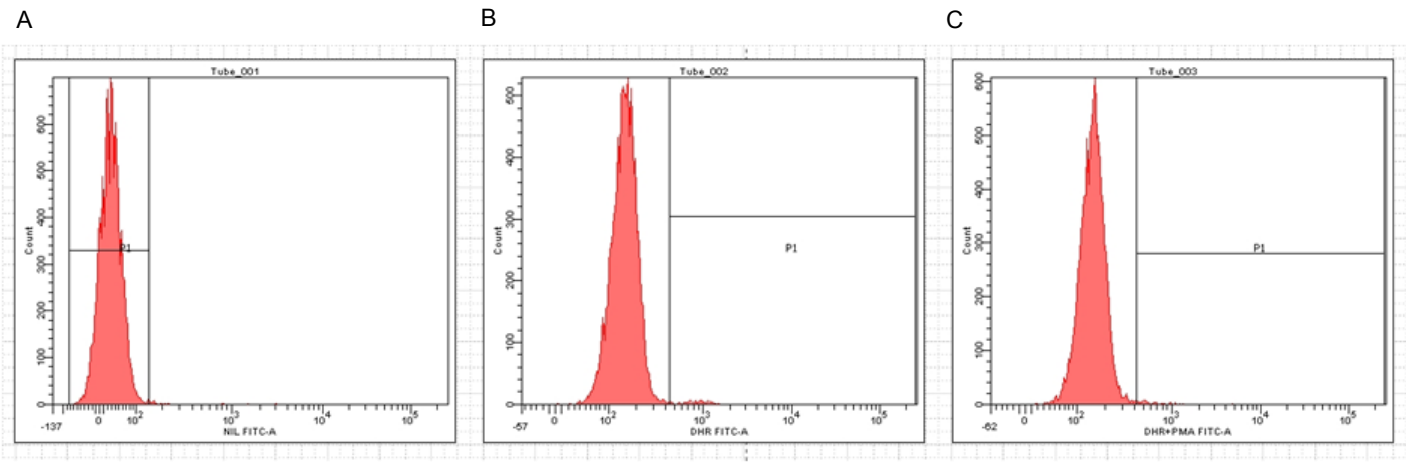

Figure 6. DHR pattern in X-linked CGD. (A) “blank”, (B) “resting” and (C) “stimulated” neutrophils.

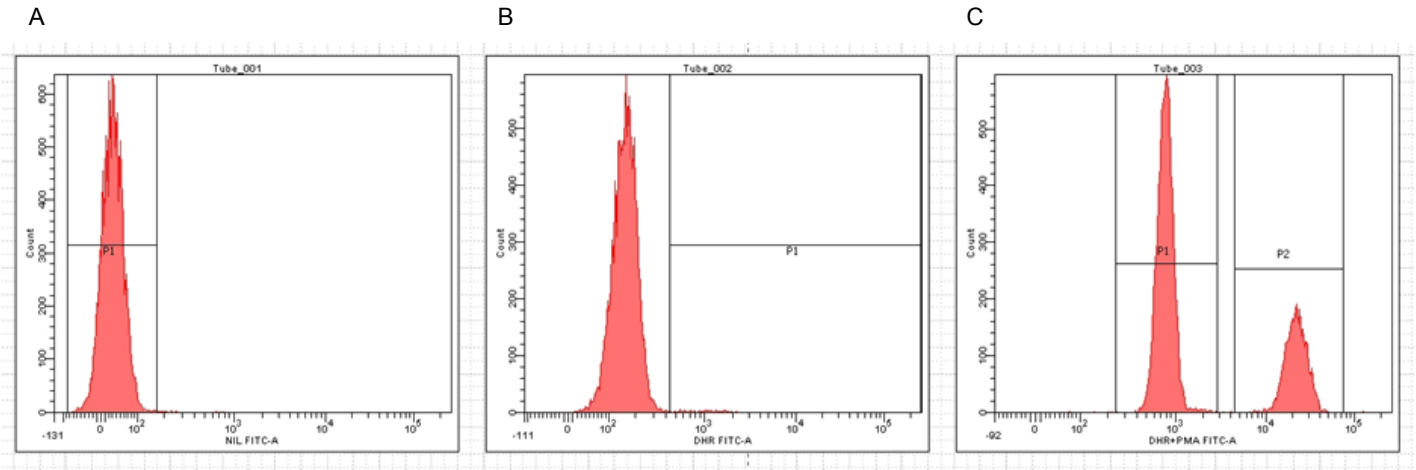

Figure 7. DHR pattern for CGD carrier state. (A) “blank”, (B) “resting” and (C) “stimulated” neutrophils.

Table 1. Results for NBT slide stain and DHR 123 flow cytometric assays.

Test #	% Glass adherent positive cells	Zymosan stimulated positive cells	Interpretation of NBT results	DHR 123 flow cytometry assay (peaks)	Interpretation of DHR results
1	>30	>90	Normal	Normal	Normal
2	>30	>90	Normal	Normal	Normal
3	>30	>90	Normal	Normal	Normal
4	>30	>90	Normal	Normal	Normal
5	>30	>90	Normal	Normal	Normal
6	0	0	Suggestive of CGD	Abnormal	Consistent with CGD
7	1	62	Suggestive of CGD	Abnormal	Consistent with CGD
8	20	42	Suggestive of CGD or carrier status	Two peaks	Possible X-linked carrier status
9	>30	>90	Normal	Normal	Normal
10	>30	>90	Normal	Normal	Normal
11	>30	>90	Normal	Normal	Normal
12	>30	>90	Normal	Normal	Normal
13	>30	>90	Normal	Normal	Normal
14	>30	>90	Normal	Normal	Normal
15	>30	>90	Normal	Normal	Normal
16	70	84	Suggestive of carrier status	Two peaks	Possible X-linked carrier status
17	>30	>90	Normal	Normal	Normal
18	>30	>90	Normal	Normal	Normal
19	>30	>90	Normal	Normal	Normal
20	>30	>90	Normal	Normal	Normal

Discussion

CGD is a rare inherited disorder of phagocytic cells that can be attributed to a variety of genetic mechanisms. The main characteristic is a defect in the enzymes that produce superoxide and other free radicals, resulting in a failure of phagocytes to destroy ingested microorganisms. Patients with CGD demonstrate vulnerability to infections caused by catalase positive organisms that are able to break down neutrophil and monocyte derived hydrogen peroxide. The lack of the formation of the superoxide free radical in CGD reduces the ability of the host's immune system to fight off infections. Most cases of CGD are inherited X-linked disorders, although examples of autosomal recessive CGD are also seen. The genetic lesions that cause CGD are variable and may include deletion, frame-shift, nonsense or missense mutations. Female carriers of the X -linked type of CGD exhibit unequal representation of both normal and mutated phagocytes as a result of lyonisation and may be either asymptomatic or have a mild form of CGD.

The activity of neutrophils in the NBT test depends upon the ability of the cells to both adhere to glass and to produce oxidative radicals. The reduction of NBT relies upon cellular NADPH oxidase and therefore the test enables an evaluation of the critical early stages of the respiratory burst (2). In the NBT "glass" slides, the adherence of the neutrophils to the glass surface in the presence of opsonin causes some neutrophils to take up and reduce the NBT. These slides allowed an evaluation of the proportion of neutrophils able to reduce NBT and produce blue formazan staining, in unstimulated cells. To assess the effect of stimulation on the oxidative burst capability of neutrophils, zymosan was used to activate neutrophils in the "stimulated" slides. Zymosan is derived from the cell wall of yeast, and in the presence of complement uptake by phagocytic cells is enhanced. Neutrophil phagocytosis of the zymosan causes activation of NADPH oxidase demonstrated by the reduction of NBT to intracellular formazan.

The results of the NBT test showed sixteen patients with normal results; however, the presence of a carrier state could not be excluded by this method on these samples. Four patients showed results outside of the normal range for the neutrophil reduction of NBT dye, by demonstrating less than 30% of the glass adherent cells with formazan staining and/or less than 90% zymosan stimulated cells showing formazan deposits. For these samples results were consistent with patterns suggestive of either a CGD carrier or disease state in the patients. Clinical correlation of laboratory test results is important and repeat testing to confirm initial findings is standard laboratory practise in such cases.

In contrast to the time consuming process of sample collection at the patient's bedside and the time taken to perform the NBT slide test, the DHR 123 assay was a quicker method to perform. The assessment of the respiratory burst by this method relies upon change in the fluorescence of resting neutrophils following stimulation. In stimulated cells increased fluorescence is indicative of the production of H_2O_2 in peripheral blood neutrophils (6). The DHR assay utilises PMA as a strong non-physiological stimulant to activate membrane-associated NADPH oxidase by enhancing protein kinase C, which in turn stimulates superoxide and hydrogen peroxide production (1,6). PMA causes a sustained respiratory burst with high levels of fluorescence seen in samples from normal individuals (Fig. 5C). PMA is the preferred means of stimulation of the respiratory burst in the DHR assay with other stimulants shown to generate smaller and more variable neutrophil responses (6). During the oxidative burst, the fluorochrome DHR 123 reacts specifically with the cellular H_2O_2 oxidising the dye to rhodamine (1). This reaction may also be dependent on the presence of cellular myeloperoxidase (2).

CD45 is a protein antigen expressed on all haemopoietic white cells and "gating" the CD45 antigen positive cells in each sample was performed as part of the DHR assay in this study. The CD45 expression together with side scatter properties (complexity of cells) allows for the better selection of peripheral blood neutrophils.

In the DHR 123 assay FACS lyse solution was used to stop the reactions and to lyse red cells while partially fixing the white cells prior to fluorescence measurement. The purpose of the inclusion of a "blank" was to check each sample for background fluorescence, while the "resting" tube was used to set the negative marker. The "stimulated" samples from the sixteen patients which produced strong fluorescence (Figure 5C) following processing would be interpreted as "Normal activity of neutrophils". Samples from the two patients that showed a lack of response to stimulation with PMA and no change in fluorescence intensity (Figure 6C), would be interpreted as "Results consistent with CGD". Two other samples showed some response with PMA and the flow histogram showed two fluorescence peaks indicating two populations of neutrophils, one with normal oxidative burst activity and the other either lacking or showing a reduced level of activity (Figure 7C). The interpretative comment provided for these patients would be "Results consistent with carrier status for X-linked CGD". All samples testing positive in the DHR assay were repeat tested as per the laboratory protocol for the assay.

In contrast to the manual NBT slide method, the DHR assay was faster to perform but it required a different skill set and a flow cytometer. The number of cells evaluated for fluorescence in the flow cytometer was set at 10,000 which greatly exceeded the 100 cells examined in the manual NBT test. The use of EDTA anticoagulated blood samples for the DHR assay has a number of advantages over samples required for the NBT test. The DHR assay eliminates the need to collect bedside patient samples making laboratory testing for CGD at Labplus available not only for hospitalised patients but also for those who are not physically onsite (e.g. outpatient clinics and community collection centres). Due to the specialised nature of this test, most medical laboratories in New Zealand refer samples to specialist testing laboratories. Sending blood samples rather than patients for testing is more convenient for the patient and more cost effective for hospitals. Samples as small as 0.5 ml of blood can be used in the DHR assay making the method ideal for studies of the respiratory burst in children, the most common age group investigated for CGD. Other advantages of the DHR assay over the NBT test include its ability to more clearly identify CGD patients and to establish the carrier state of the X-linked forms of the condition.

Limitations on the successful testing for CGD via the DHR method relate to the relatively short lifespan of PMNs. Samples for testing must arrive in the laboratory on the same day of collection which could have implications for testing from outside of the Auckland area. For these the parallel testing of a "transport control" from a normal patient may help to validate patient results. In addition to this, patients with inflammatory states or current infections are negative indicators for the use of the DHR assay for CGD screening. These conditions can make the DHR results difficult to interpret requiring repeat testing following the cessation of inflammation or infection.

Conclusions

In this study, the qualitative NBT slide test and the quantitative DHR 123 flow cytometric assays were compared for their diagnostic performance for the diagnosis of CGD. Both methods measured the ability of neutrophils to mount a neutrophil respiratory burst and results of the NBT test and those from the DHR fluorescence test showed good correlation. The results from both methods lead to similar clinical interpretations, the only exception being that the NBT was not able to identify or exclude carrier states of X-linked CGD.

The qualitative NBT slide test is time consuming and labour intensive, requiring technical skills that include the visual inspection of a limited number of peripheral blood neutrophils. Samples used for the NBT test require bedside blood collection, restricting testing to inpatients bringing inconvenience to patients and increased costs. The DHR method is a quantitative measurement of the respiratory burst and is an assay that is quick to perform requiring only small volumes of EDTA anticoagulated blood. The DHR assay provides distinctive histogram patterns that make the interpretation of results less subjective and is able to differentiate between X-linked CGD and X-linked CGD carrier patients. At LabPlus, the DHR assay has become the method of choice for screening and/or diagnosis of patients for CGD.

This study was only able to include a small number of CGD and carrier state patient samples because of the scarcity of cases and the low number of requests for CGD screening tests. The original study was also not able to test the performance of the DHR assay against samples from the less common autosomal recessive form of CGD. Since the original study, three cases of autosomal recessive CGD have been tested using the DHR assay with the results obtained consistent with previously reported NBT results on these patients.

Acknowledgements
This article reports the work undertaken by staff at Labplus and was presented by Geri Dimitrova during her 4th year placement in Haematology at LabPlus. The work formed part of the requirement of the Massey BMLSc. The authors declare no conflicts of interest.

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References

1. Walrand S, Valeix S, Rodriguez C, Ligot P, Chassagne J, Vasson M P. Flow cytometry study of polymorphonuclear neutrophil oxidative burst: a comparison of three fluorescent probes. *Clinica Chimica Acta* 2003; 331: 103–110.
2. Richardson MP, Ayliffe MJ, Helbert M, Davies EG. A simple flow cytometry assay using dihydrorhodamine for the measurement of the neutrophil respiratory burst in whole blood: comparison with the quantitative nitroblue tetrazolium test. *J Immunol Methods* 1998; 219: 187–193.
3. Rae J, Newburger PE, Dinauer MC, Noack D, Hopkins PJ, Kurto R, et al. X-linked chronic granulomatous disease: mutations in the CYBB gene encoding the gp91 -phox component of respiratory-burst oxidase. *Am J Hum Genet* 1998: 62: 1320–1331.
4. Metcalf JA, Gallin JI, Nauseef WM, Root RK. Laboratory Manual of Neutrophil Function. Lippincott-Raven, New York, 1986; pp. 100- 103.
5. Gifford RH, Malawista SE. A simple rapid method for detecting chronic granulomatous disease of childhood. *J Lab Clin Med* 1970; 75: 511-519.
6. van Eeden SF, Klut ME, Walker BA, Hogg JC. The use of flow cytometry to measure neutrophil function. *J Immunol Methods* 1999; 232: 23–43.

A comparison of four commercial chromogenic media and blood agar for the isolation and preliminary identification of *Streptococcus agalactiae* from vaginal swabs

Clare Tibbs and Julie Creighton

Abstract

Aim: To evaluate the performance of four commercial chromogenic media for the detection of GBS in vaginal swabs from pregnant women. The media was compared with blood agar and was evaluated in terms of sensitivity, specificity, ease of use and cost.

Methods: 100 vaginal swabs, collected during April and May 2011, were included in the study. The swabs were pre-enriched in LIM broth for 16-24 hours, before a 10µL loop of broth was sub-cultured onto blood agar and each of the chromogenic media. CHROMagar™ StrepB, bioMérieux ChromID, Oxoid Granada, and Bio-Rad StrepB Select, were compared with 5% Columbia blood agar and routine laboratory detection. Incubation of plates and interpretation of bacterial growth was in accordance with the manufacturers' guidelines.

Results: Bio-Rad StrepB Select and bioMerieux ChromID were the best performing media with 100% and 96% sensitivity and 91% and 92% specificity, respectively. CHROMagar™ StrepB and Oxoid Granada were less sensitive (88% and 72%), with no improved performance over blood agar.

Conclusion: bioMérieux ChromID was chosen for introduction into routine use, due to its excellent sensitivity and easier differentiation of false positive growth.

Keywords: *Streptococcus agalactiae*, Group B Strep, vaginal swab, LIM broth, Bio-Rad StrepB Select, bioMerieux ChromID, CHROMagar™ StrepB, Oxoid Granada.

N Z J Med Lab Sci 2013; 67: 52-55

Introduction

Streptococcus agalactiae (Group B Strep (GBS)) remains a leading cause of invasive disease in neonates. GBS can cause septicaemia, meningitis and pneumonia in the neonate, with resulting high morbidity and mortality rates. Neonates are infected by perinatal transmission and can become ill within hours of becoming infected, or take up to 5 days before symptoms start to show (1).

S. agalactiae is a Gram positive coccus that is weakly beta haemolytic with a dull grey colonial appearance on 5% Columbia sheep blood agar. Most colony sizes are 1-2mm and colonies can be hard to detect if there is heavy growth of other commensal bacteria present (2,3,4). Normal vaginal flora includes *Staphylococcus species*, lactobacillus, *Enterococcus species*, α-haemolytic, β-haemolytic and non-haemolytic streptococcus.

Laboratory confirmation of suspect GBS colonies is commonly performed by Lancefield grouping, whereby *Streptococcus species* are classified into serologic groups based on their cell wall antigenic properties. *S. agalactiae* belongs to Lancefield Group B.

Asymptomatic GBS vaginal or rectal colonisation occurs in approximately 10-40% of women. Pregnant women colonised with GBS risk maternal transmission during labour. It is in this group of women that antibiotic prophylaxis is most effective (1).

The United States Centers for Disease Control and Prevention (CDC) recommend for women between 35-37 weeks of gestation to be screened for GBS carriage. However, the New Zealand College of Midwives advocate a risk-based approach, whereby only women deemed to have high risk factors, such as premature rupture of the membranes and preterm delivery, should be screened or given antibiotic prophylaxis. For screening purposes, high vaginal swabs (HVS) or rectal swabs are used, with collection usually carried out by either a registered nurse or a midwife.

Common problems with the laboratory detection of GBS include low colony forming units of GBS in some samples, overgrowth of normal vaginal flora and non-haemolytic GBS colonies. In addition, if GBS is present with non-typical colony morphology it can be difficult to detect or missed, particularly on blood agar (2,3,4). To detect low numbers of organisms, swabs should be pre-enriched in either Todd-Hewitt broth or LIM broth, before agar plate culture (5). Specific GBS selective chromogenic media have been developed by various companies in order to overcome some of the detection problems and to improve isolation rates.

The aim of this study was to evaluate the performance of four chromogenic media for the detection of GBS in 100 high vaginal swabs from pregnant women. The four chromogenic media were compared with blood agar and evaluated in terms of sensitivity, specificity, ease of use, and cost.

Method and materials

100 vaginal swabs, collected from pregnant women during April and May 2011, were included in the study. Swabs were taken during either routine outpatient antenatal screening or pre delivery at Christchurch Hospital. Data on pregnancy gestation was not collected. Swabs were collected into transport media and processed at Canterbury Health Laboratories within 24 hours of collection. Routine laboratory processing was performed independently of the study.

Routine laboratory protocol for HVS analysis consisted of inoculation onto blood agar and Chocolate/Thayer Martin agar, with a wet prep for *Trichomonas vaginalis* and a Gram stain. The swabs were then broken off into LIM broth (Fort Richard Laboratories, Auckland), for the selective enrichment of GBS. The LIM broth is an enriched brain heart infusion base with added nalidixic acid and colistin to suppress the growth of gram negative bacteria (5). LIM broth was incubated for 16-24 hours at 36˚C, 5% CO_2, before being sub-cultured with a 10µL loop onto blood agar. The blood agar plates were incubated for 16-24 hours at 36˚C, in 5% CO_2. The plates were then read for GBS, looking for colonies that have typical GBS morphology (4).

Four different chromogenic agar culture plates: CHROMagar™ StrepB, ChromID, Oxoid Granada, and StrepB Select, were compared with 5% Columbia blood agar and routine laboratory detection. All plates were inoculated with a 10µl suspension of LIM broth.

CHROMagar™ StrepB (Fort Richard Laboratories, Auckland) is a selective agar that inhibits most bacteria and allows for differentiation of GBS, which produces a mauve colour (Fig 1). ChromID (bioMérieux, New Zealand) produces colonies that are pink to red in colour. Other bacteria are either inhibited by the media or are blue to green pigmented (Fig 2). Oxoid Granada (Thermofisher Scientific, Auckland) is a clear media, producing orange pigmented colonies in anaerobic conditions (Fig 3). StrepB Select (Bio-Rad New Zealand) produces distinctive blue colonies on an opaque media (Fig 4).

Blood agar plates were incubated for 18 hours at 36˚C with 5% CO_2. CHROMagar™ StrepB, bioMérieux ChromID and Bio-Rad StrepB Select were incubated at 36˚C in ambient air conditions. Oxoid Granada agar plates were incubated for 18 hours at 36˚C in anaerobic conditions. After overnight incubation, all plates were read in accordance with the manufactures' guidelines.

Typical GBS colonies were confirmed by ProLab Diagnostics Strep latex grouping kit (Ngaio Diagnostics, Nelson). False positive colonies were investigated using standard laboratory methods. The isolation of GBS from any of the media used (chromogenic or blood) was considered to be a positive sample, and failure to detect GBS on any of the other media was considered to be a false negative.

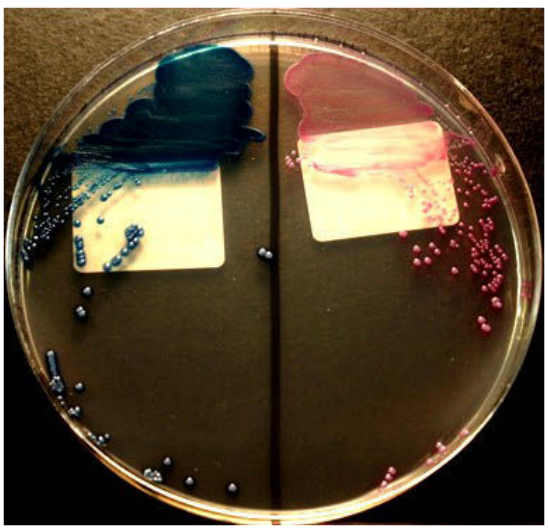

Figure 1. CHROMagar StrepB, with Enterococcus species on the left hand side of the plate and GBS on the right hand side of the plate showing a light pink colouration.

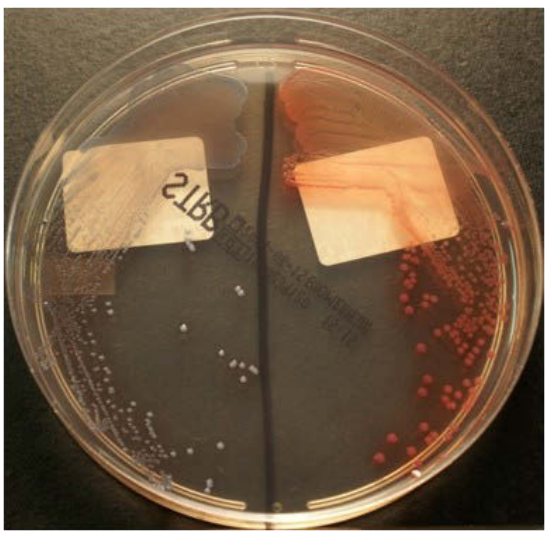

Figure 2. bioMérieux ChromID, with Enterococcus species on the left hand side as light blue, and GBS on the right hand side as pink pigmented colonies.

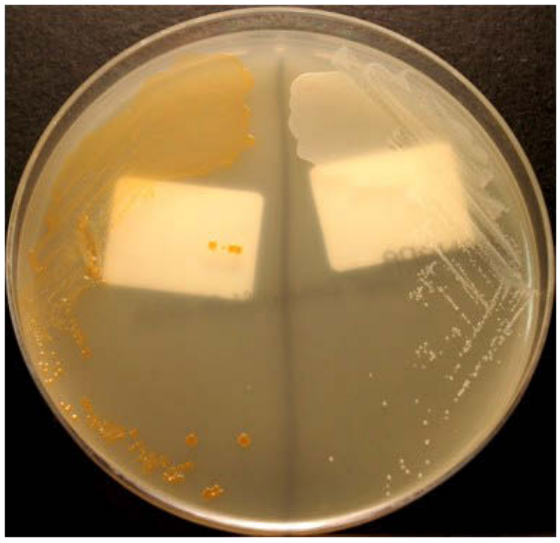

Figure 3. Oxoid GRANADA, with GBS on the left hand side of the plate exhibiting yellow-orange pigmentation. On the right hand side is non-inhibited growth which is not GBS.

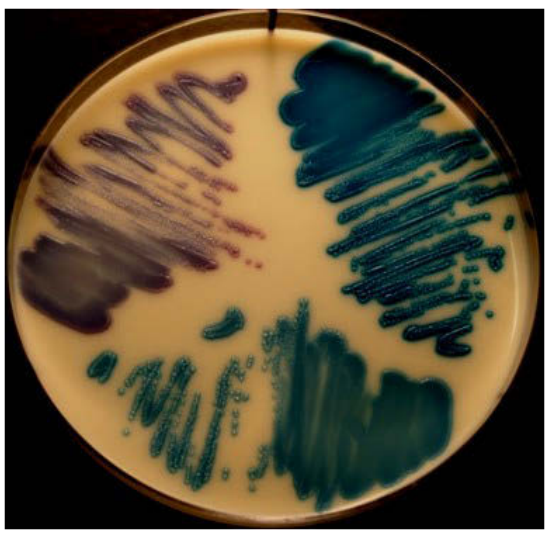

Figure 4. Bio-Rad StrepB SELECT, with lavender colonies indicating non-GBS growth and two different shades of blue representing GBS colonies.

Results

After testing all suspect colonies from the chromogenic agar, there were 25 positive GBS cultures. Bio-Rad StrepB Select detected all 25 isolates, bioMérieux ChromID detected 24 GBS, CHROMagar™ StrepB detected 22 GBS and Oxoid Granada was the least sensitive detecting only 18 GBS. In comparison there were 22 GBS isolated from blood agar but only 20 were detected by routine laboratory testing. Results are listed in Table 1. The population prevalence for GBS in this study is 25%, which is similar to the population prevalence reported by Hickman et al. (1).

Table 1. Media performance

Media	True Positives	False Positives	False Negatives	Sensitivity	Specificity
Blood agar	22	4	3	88%	95%
CHROMagar™ StrepB	22	15	3	88%	83%
bioMérieux ChromID	24	6	1	96%	92%
Oxoid Granada	18	3	7	72%	96%
Bio-Rad StrepB Select	25	7	0	100%	91%
Routine laboratory procedures	20	NR	NR	NR	NR

All of the chromogenic media tested gave false positive results. Colonies were further investigated to reveal the identity of false positive organisms, with staphylococci and streptococci being the major culprits. Results are detailed in Table 2.

Table 2. Number of false positive growths

	Blood agar	**CHROMagar**	**bioMérieux**	**Oxoid**	**Bio-Rad**
CNS	0	1	0	3	0
α-haemolytic streptococcus	0	6	2	1	3
B-haemolytic streptococcus	4	2	2	0	1
Non-haemolytic streptococcus	0	8	1	0	1
Staphylococcus aureus	0	0	1	0	2

In terms of specificity, the Oxoid Granada media had the least number of false positive isolates. However this was counteracted by its lack of sensitivity. Bio-Rad StrepB Select and bioMerieux ChromID had a small number of false positive isolates that required further confirmation tests. CHROMagar™ StrepB had a high number of false positive isolates, giving it the lowest specificity. In comparison, blood agar had only 4 false positive isolates.

Discussion

As expected, blood agar missed several GBS positive cultures. This was mainly due to overgrowth of normal vaginal flora. However, two of the chromogenic agars also lacked sensitivity. Only two of the trial media (bioMérieux ChromID and Bio-Rad StrepB Select) out performed blood agar.

The Bio-Rad StrepB Select media proved to be the most sensitive for GBS at 100% but had a number of false positives with colonies looking very similar to GBS colonies in terms of colour and colony size. bioMérieux ChromID was 96% sensitive for the detection of GBS. Most of the false positives from this agar were smaller than 1mm or the colour was lighter than a true GBS. With experience, staff will be able to easily distinguish a true positive colony, thus reducing unnecessary follow up work.

FRL CHROMagar™ StrepB agar did not perform well in this study. The sensitivity was only 88% and it had a high rate of false positives, with normal flora difficult to distinguish from GBS. Likewise, Oxoid Granada agar was also a poor performer in terms of sensitivity, missing 28% of GBS; although it had a low false positive rate. We found both of these media unsuitable for clinical diagnostic testing for GBS in pregnant women.

When evaluating new methods it is important to also look at costs. Three of the chromogenic media (Bio-Rad StrepB Select, bioMerieux ChromID, and FRL CHROMagar) are of similar price per plate. However, a high false positive rate adds considerable additional cost and time. For this reason, FRL CHROMagar is more costly to use than Bio-Rad StrepB Select or bioMerieux ChromID. Oxoid Granada was the most expensive media trialled.

Conclusion

In this small study we have found that the two best performing chromogenic media were bioMérieux ChromID and Bio-Rad StrepB Select. This was based on a high sensitivity for GBS, with a low false positive rate and similar plate price. The workup required for these media was lower than for the other two media tested. BioMérieux ChromID was chosen for introduction into routine use at Canterbury Health Laboratories due to its excellent sensitivity and reader ability to easily distinguish false positive growth.

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References

1. Hickman ME, Rench MA, Ferrieri P, Baker CJ. Changing epidemiology of group B streptococcal colonization. *Pediatrics* 1999; 104 (2 Pt 1): 203-209.
2. Perry JD, Oliver M, Nicholson A, Wright J, Gould FK. Evaluation of a new chromogenic agar medium for isolation and identification of Group B streptococci. *Lett Appl Microbiol* 2006; 43: 615-618.
3. Poisson DM, Chandermerie M, Guinard J, Evrard ML, Naydenova D, Mesnard L. Evaluation of CHROMagar StrepB: A new chromogenic agar medium for aerobic detection of Group B Streptococci in perinatal samples. *J Microbiol Methods* 2010; 82: 238-242.
4. Poisson DM, Evrard ML, Freneaux C, Vivès MI., Mesnard L. Evaluation of CHROMagar™ StrepB agar, an aerobic chromogenic medium for perpartum vagina/rectal Group B Streptococcus screening. *J Microbiol Methods* 2011; 84: 490-491.
5. Rauen NC, Wesenberg EM, Cartwright CP. Comparison of selective and non-selective enrichment broth media for the detection of vaginal and anorectal colonization with group B streptococcus. 2005; 51: 9-12.

Eosinophilia in a patient with lung adenocarcinoma - a case study

Jun (Rebecca) Lu and Craig Rodgers

Abstract

Eosinophilia, or an elevated eosinophil count in peripheral blood, is frequently seen in people with allergic reactions and parasitic infections. However, its occurrence in patients with solid tumors is relatively low. Full blood count analysis of an 86 year old patient revealed a marked eosinophilia of 42.3 x 10^9/L, who had previously been diagnosed with lung adenocarcinoma with metastasis into bone marrow and lymph nodes. It is suggested by studies that over-expression of cytokines, namely granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-5 (IL-5) could be involved in the process of eosinophilia development, due to their direct effects on eosinophils. Cytokine production by tumor cells, tumor necrosis and metastasis into bone marrow are considered to be the main causes of eosinophilia in patients with cancers.

Key words: eosinophilia, lung adenocarcinoma, granulocyte-macrophage colony-stimulating factor, interleukin-5.

N Z J Med Lab Sci 2013; 67: 56-57

Introduction

The eosinophil is a type of granulocyte and usually accounts for a very small proportion of total white cell count in peripheral blood. In our laboratory, an absolute eosinophil count of greater than 2.0×10^9/L is defined as marked eosinophilia. Eosinophilia, particularly marked eosinophilia, is a phenomenon commonly associated with allergy, parasitic infestations and drug hypersensitivities, but rarely linked to pre-existing solid tumors. We present here a case of a patient diagnosed with lung adenocarcinoma who then developed marked eosinophilia.

Case report

In July 2010, Mrs H, an 86 year old female, had her routine check-up performed at a rest home and blood samples were also taken. The full blood count analysis revealed remarkable abnormalities, including a haemoglobin (Hb) of 65 g/L (reference range: 115-155 g/L), mean cell volume (MCV) of 115 fl (reference range: 81-98 fl), platelet count of 48 x 10^9/L (reference range: 150-430 x 10^9/L) and a total white cell count of 61.3 x 10^9/L (reference range: 4.0-11.0 x 10^9/L). The DIFF scattergram from the XE2100 analyser contained a large grey-out area where the population of eosinophils normally locates (Figure 1). The analyser stated that 93% of the white cells were eosinophils. A blood film was then made to perform a manual white cell differential and examine cellular morphology. Sixty-nine percent of the white cells were identified as eosinophils, giving an absolute eosinophil count of 42.3 x 10^9/L. As shown in Figure 2, a proportion of the eosinophils appeared hypogranular. There was a left shift of neutrophils which also had features of toxic change. Moreover, occasional metamyelocytes and nucleated red blood cells were also noted.

The patient presented in the Emergency Department four hours later and similar full blood count results were obtained, where eosinophils accounted for 46% of the total white cell count. It was also found that the patient had an elevated Troponin T result and deteriorating renal function. The blood film was then reviewed by a haematologist who commented that it was consistent with secondary eosinophilia and leukamoid reaction, whilst bone marrow infiltration might also be involved. After her admission into the ward, she was transfused with two units of red cells and given prednisolone.

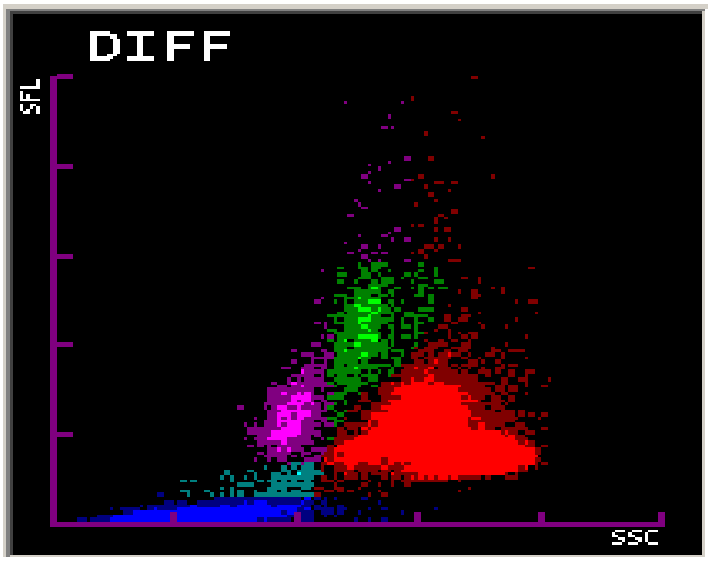

Figure 1. DIFF scattergram taken from XE2100 on the patient.

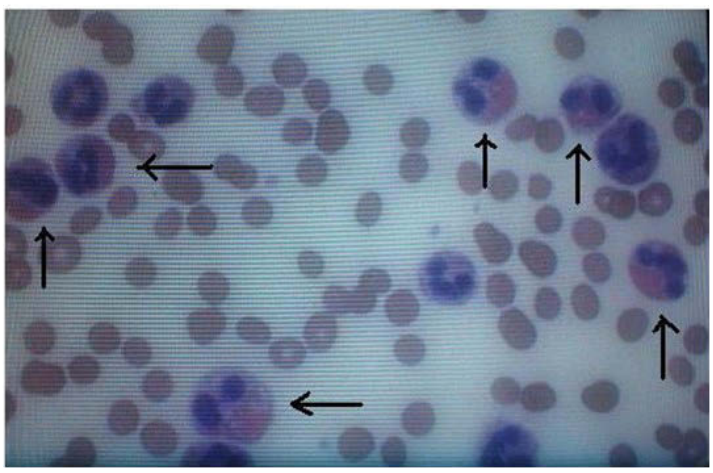

Figure 2. Image of the patient's blood film showing six eosinophils in a single field.

Further research on the patient revealed that Mrs H had pernicious anaemia since the age of 30. Since late 2007, the patient had developed mild macrocytic anaemia with a borderline low platelet count. In early 2009, she was diagnosed with malignant neoplasm of the lung, specifically adenocarcinoma of her right lung, using bronchoscopy and treated with radiotherapy. Three months later, Mrs H developed an episode of stroke. During her hospital admission, secondary neoplasm of lymph nodes, bone and bone marrow, mainly in the left pelvis, was confirmed which was then followed by another course of radiotherapy. Over the following years, she had several top-up transfusions and developed moderate thrombocytopenia of 80-100×10^9/L. Until the presentation of this event, an elevated white cell count (22.7-36.3×10^9/L) and eosinophilia ranging from 14% to 36% were frequent findings in her blood count.

Discussion

Eosinophilia in peripheral blood is often seen in individuals with allergy, parasitic infection, eosinophilic leukaemia and hypereosinophilic syndrome (1-4). The persistence of eosinophilia for an extended period of time can cause damage to various body tissues, mainly due to release of the contents of cytoplasmic granules in eosinophils (4). As a result, some eosinophils may appear degranulated or even agranular in the blood film (5). However, the occurrence of eosinophilia in people with solid tumors is low (3).

Several studies suggest that a number of cytokines are involved in the development of blood hypereosinophilia in cancer patients, particularly interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (1-3,6,7). Both cytokines play an important role in the production, activation and survival of eosinophils in the bone marrow and peripheral blood, as well as the enhancement of cellular functions of eosinophils in peripheral circulation (6,7).

GM-CSF is a growth factor that stimulates the development of myeloid cells, or myelopoiesis, via its receptors, and is synthesized mainly by stromal cells in the bone marrow (8). As an alternative source of GM-CSF in some lung cancer patients, its secretion by tumor cells has also been detected (1, 2, 8). Subsequently, proliferation of white cells is stimulated by exogenous GM-CSF resulting in leukocytosis as well as eosinophilia (1,7). IL-5, a cytokine normally produced by T cells, acts specifically on eosinophilic cell lineage allowing its activation (3,7). Additionally, the presence of IL-5 enhances cytotoxic function of the eosinophils against tumor cells in cancer patient (3).

Three major factors are thought to be responsible for the development of eosinophilia in patients with known cancers with or without metastasis (3). Locally produced active cytokines by tumor cells have direct effects on eosinophils and their functions (1.3). Tumor necrosis, as a result of treatment for cancers, such as radiotherapy, can lead to eosinophilia (3). It is usually a poor prognosis indicating possible persistence of tumor after treatment (9). Metastasis from the primary tumor, especially into bone marrow, results in bone marrow stimulation mediated by various cytokines and thus, eosinophilia in the peripheral circulation (3). For our patient, all theories above could be accountable.

Bone marrow aspirate is of limited use for differentiating between eosinophilia due to chronic eosinophilic leukaemia and paraneoplastic syndrome, relating to the underlying malignancy, since the hypercellular feature is common to both conditions. However, a measurement of GM-CSF level by immunoassay in pleural fluid in patients with lung cancer is valuable (1). Intracellular IL-5 in tumor cells determined by immunohistochemistry may also be used as a supplementary test (3). On the other hand, a chromosomal abnormality or molecular mutation is often one of the major findings in cases of chronic eosinophilic leukaemia (4). Unfortunately, our patient passed away two days after admission and no further analysis was carried out.

Conclusion

Besides the common eosinophilia occurring during allergic reactions and parasitic manifestations, it is likely that some patients with solid tumors will develop eosinophilia as one of the paraneoplastic syndromes, which is mediated by the actions of cytokines, especially when treatment-related tumor necrosis and/or metastasis into bone marrow have also been confirmed.

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References

1. Sawyers CL, Golde DW, Quan S, Nimer SD. Production of granulocyte-macrophage colony-stimulating factor in two patients with lung cancer, leukocytosis , and eosinophilia. *Cancer* 1992; 69: 1342-1346.
2. El-Osta H, El-Haddad P, Nabbout N. Lung carcinoma associated with excessive eosinophilia. *J Clin Oncol* 2008; 26: 3456-3457.
3. Pandit R, Scholnik A, Wulfekuhler L, Dimitrov N. Non-small-cell lung cancer associated with excessive eosinophilia and secretion of interleukin-5 as a paraneoplastic syndrome. *Am J Hematol* 2007; 82: 234-237.
4. Hoffbrand AV, Moss PAH. Essential Haematology. 6th ed. Wiley-Blackwell, UK, 2011.
5. Bain BJ. Blood Cells: A Practical Guide. 4th ed. Blackwell Publishing, Australia, 2006.
6. Fridlender ZG, Simon HU, Shalit M. Metastatic carcinoma presenting with concomitant eosinophilia and thromboembolism. *Am J Med Sci* 2003; 326: 98-101.
7. Sun Q, Jones K, McClure B, Cambareri B, Zacharakis B, Iversen PO, et al. Simultaneous antagonism of interleukin-5, granulocyte-macrophage colony-stimulating factor, and interleukin-3 stimulation of human eosinophils by targeting the common cytokine binding site of their receptors. *Blood* 1999; 94: 1943-1951.
8. Watanabe M, Ono K, Ozeki Y, Tanaka S, Aida S, Okuno Y. Production of granulocyte-macrophage colony-stimulating factor in a patient with metastatic chest wall large cell carcinoma. *Jpn J Clin Oncol* 1998; 28: 559-562.
9. Lowe D, Jorizzo J, Hutt MS. Tumour-associated eosinophilia: a review. *J Clin Pathol* 1981; 34: 1343-1348.

TWENTY FOURTH ANNUAL NICE WEEKEND

17—19 May 2013

Bayview Wairakei Resort

The 24rd National Immunohaematology Continuing Education (NICE) weekend was held 17th to 19th of May at the Bayview Wairakei Resort in Taupo. All attendees at NICE are required to give a two – five minute presentation or prepare a poster. NICE provides a supportive atmosphere for those who are not so familiar or comfortable with presenting to "give it a go", while being able to learn from the more seasoned presenters amongst us. It is also a great way to network and catch up with our fellow transfusion science colleagues.

Over the weekend we were treated to 47 fantastic presentations and were able to view and discuss 11 poster presentations. All presenters did a fantastic job. The general consensus was that the standard of presentations delivered over the weekend was very high and a wide variety of presentation topics were covered.

Congratulations to all our attendees who took away awards for their presentations/poster:

- The Abbot Award for Best Overall Presenter went to Jared Pratt (Pathlab BOP) for his talk – **I can feel it in my bones**
- The Ortho Clinical Diagnostics Award for Most Promising Transfusion Scientist went to Abby Clayton (NZBS Wellington) for her talk on her MSc Research Project – **Comparison of Methods to Assess Postpartum Haemorrhage in Rh (D) Negative Women**
- The Pharmaco Award for Best Poster went to Alexandra Shafer (NZBS Auckland) for her poster **Transfusion Support of a Malaria Patient**
- The bioCSL award for a New Zealand attendee to attend NICE Australia was this year won by Eamon Karalus (T-Lab Ltd Gisborne)
- The NZIMLS Award for the Best First Time Speaker — a presenter who has never attended NICE weekend before, giving them the title of NICEst Virgin. This went to Sandya Arunachalam (AUT Student) for her talk explaining the relationship between **Blood Group Antigens and Infectious Disease**
- Congratulations must also go to Tony Morgan (Hawkes Bay DHB) and Kendall Wills (Medlab Nelson) who received special mention for their presentations. Kendall introduced and impressed NICE attendees with the PechaKucha 20x20 presentation style. PechaKucha 20x20 is a simple presentation format where you show 20 images, each for 20 seconds. The images advance automatically and you talk along to the images. This presentation style is definitely worth investigating.

The statistics: NICE 2013 was attended by 80 people in total including 19 trade representatives, one sponsored Student, one sponsored convener, 59 delegates including one participating TMS and one Australian visitor. Of these 49 were NZIMLS members and 31 where non-NZIMLS members.

The theme for this year's NICE weekend was "Once Upon a Time", which was carried over to a spell binding fancy dress dinner. All attendees looked fantastic in a wide variety of costumes and a fun evening was had by all.

We must also acknowledge the support of our sponsors who continue to make this weekend possible:

- MedTel NZ Ltd
- Ortho Clinical Diagnostics
- BioRad Laboratories
- Roche
- Grifols
- bioCSL
- Abbott Diagnostics Division
- Pharmaco (NZ) Ltd
- TempRecord International Ltd
- Abacus – ALS
- New Zealand Blood Service

A huge thank you on behalf of all must be extended to our amazing NICE Convenors – Grace Agustin and Raewyn Cameron. These ladies delivered another spectacular educational weekend. So on behalf of the TSSIG and the wider NICE group I would like to extend a huge Thank You. We look forward to celebrating our 25th Weekend with a silver birthday bash on the 23-25th May 2014.

Prizewinners: Eamon Karalus (NICE Australia winner), Jared Pratt (Best Presentation), Alex Shafer (Best Poster), Sandya Arunachalam (NICEst Virgin)
Absent: Abby Clayton (Most Promising Transfusion Scientist)

Melissa May

Haematology Special Interest Group Meeting

March 2013

The Haematology SIG meeting was held 2nd March 2013 in the Napier War Memorial Conference Centre. There were 49 registered delegates with 26 attending the dinner at the Scenic Hotel Te Pania following the meeting.

There was a full and varied scientific programme incorporating all aspects of haematology, coagulation and transfusion medicine. Topics ranged from case studies through to method reviews and in-house studies. Dr Elayne Knottenbelt (Consultant Haematologist, Midcentral DHB) also presented a comprehensive morphology review of haematological emergencies.

The Best Overall Presenter award was won by Jacquie Case (Middlemore Hospital) who presented an interesting case study about malaria and the use of ICT cards.

There has been an amendment to the Best First Time Presenter award initially announced on the day. This has now been awarded to Joanna Mucznik (Pathlab Hamilton) who presented on the problems associated with platelet clumping and estimation.

It was challenging to be able to fill the programme with enough advance notice to enable the programme to appear on the NZIMLS website. Many laboratories were unable to attend and/or present for a variety of reasons. However, the feedback has been positive and the facilities provided by the Napier War Memorial Conference Centre were to a high standard. The dinner went well and the food was excellent.

I would like to again thank the sponsors – Bio-Rad Laboratories, Stago, Siemens, Beckman Coulter, Roche and the NZIMLS.

The convener for the next Haematology SIG meeting is to be decided.

Sarah Hardingham
Haematology SIG Convener 2013

2012 CPD AUDIT FINAL REPORT OF AUDIT FINDINGS

The 2012 Continuing Professional Development (CPD) audit was conducted by Walker Consulting in a timeframe equivalent to that of previous years, which spanned the Christmas 2012 and New Year 2013 holiday period.

The selected practitioners were advised eight weeks in advance of their need to submit relevant information by not later than 15th January 2012.

81.3% of respondents sent in all the correct documentation and no further follow-up for these individuals was required.

18.7% of all respondents were sent at least one email to request further information that would enable validation of some of the points they claimed.

On 18th December 2012 Walker Consulting sent reminder emails to the 159 practitioners who had yet to respond. On 9th January 2013 final reminder emails were sent to the 59 practitioners who had yet to respond. On the 22nd January 2013 8 practitioners who had not responded and who had not been excused for a valid reason were contacted by phone. Eventually responses were received from all but 2 practitioners.

Details of points claimed and points validated for individual respondents are included in the spreadsheet presented with this report ("CPD Report 2012 for Medical Laboratory Science Council of New Zealand" attached).

A summary of comments arising from this data is as follows:

- A more detailed audit advice letter that reiterated the need to present adequate supporting information for previously problematic categories resulted in a much improved level of compliance from most auditees in many categories.
- Absence of satisfactory evidence of annual competency was more common in 2012 than in previous years.
- Lack of notes, synopsis or conference report to accompany category 6 claims was also relatively common in 2012.

NZIMLS CPD Audit 1st January 2012 to 31st December 2012 Statistical Summary

Total number of enrolled practitioners:	1678	(as at 31/12/2011)
Total number of enrolled practitioners selected for audit:	246	(14.7% of total enrolments)
Total number of respondents:	230	(13.7% of total enrolments)
Total number of respondents requiring no follow-up:	187	(81.3% of those audited)
Total number of respondents by 16/01/2013:	226	(98.3% of those audited)
Late responses (received after 20/01/2013):	3	(1.3% of those audited)
Late incomplete:	1	(0.4% of those audited)
Total number of non-respondents with a valid reason given (Refer to Table 1):	13	(5.2% of those audited)
Total number of non-respondents with no reason given:	5	(2.0% of those audited)
Total points claimed by all respondents:	28520	
Total points validated for all respondents:	28423	(99.6% of points claimed)
Average points claimed per respondent:	124.0	
Average points validated per respondent:	123.6	
Respondents not claiming points in Category 1: (Compulsory section) after follow up	1	(0.4% of respondents)

Table 1: Summary of reasons for lack of response (20)

Non-practising	7	
Retired	3	
Unable to be contacted	3	(includes out of NZ)
Maternity Leave	2	
Excused	1	
Incomplete response received	2	
Response advised, not received	2	

Table 2: Summary of points validated

Note: Details of the CPD points categories are as described on the NZIMLS website "Competence and Professional Development".

Category	Points validated	% of total points validated
1	13620	47.92
2	501	1.76
3	150	0.53
4	316.5	1.11
5	1241	4.37
6	5514.5	19.40
7	450	1.58
8	690	2.43
9	130	0.46
10	989	3.48
11	219	0.77
12	691	2.43
13	0	0.00
14	2172	7.64
15	1444	5.08
16	83	0.29
17	50	0.18
18	162	0.57
Total	28423	100.00

Final report presented on 11 March 2013
Walker Consulting.

PRE ANALYTICAL SERVICES SPECIAL INTEREST GROUP PAS SIG SEMINAR 2013

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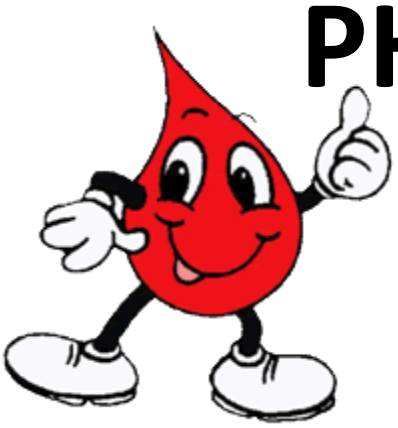

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&

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REGISTRATION + COFFEE 9:30am ● SEMINAR BEGINS AT 10:00am ● FINISH AT 4:3Opm

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Online registrations available www.nzimls.org.nz

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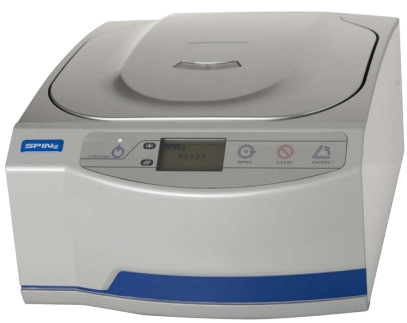

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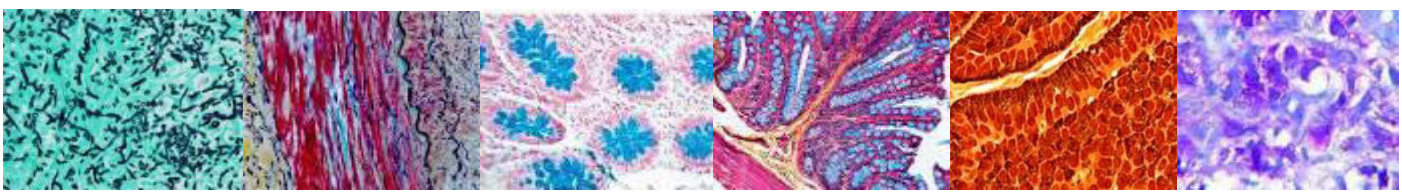
Histology SIG
Nelson 2013



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MEDLAB South

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Contact: alannah.giles@nmdhb.govt.nz

Mortuary SIG Seminar
9 November 2013
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Dunedin Hospital, Great King Street, Dunedin

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The Anatomy Department
Otago Medical School

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Contact for presentations: bill.little@southerndhb.govt.nz

Spring into Christchurch

For the 2013 Immunology SIG

Saturday 23rd November

Copthorne Hotel Commodore

Memorial Avenue

Christchurch

Registration and coffee from 9.00am

Scientific Programme 10.00am – 5.00pm

Dinner 7.00pm

Presentations required.

Please contact: Angela Horridge at
angela.horridge@sclabs.co.nz

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Courses held at the Centre

Haematology 2013

In March of this year, Phil Wakem, our PPTC manager and haematology technical consultant, provided a haematology and blood film examination course to Pacific students nominated to attend, and once again this proved to be a great success. This course provided trainees with guidelines for the objective microscopic evaluation of white cells, red cells and platelets in both health and disease.

Trainees were introduced to the workings of the microscope in terms of correct operation, correct use of objectives, and essential maintenance. They learnt the principles of Romanowsky staining, the preparation of stains and buffers, causes of inconsistent staining quality and the correct staining techniques used in the identification of malarial parasites. Students were introduced to the blood film in terms of sample quality, the effects of anticoagulants, the correct technique in blood film making, morphological artefacts, buffy coat preparations, and the correct storage of blood films. Students also learnt extensively the correlation of blood film findings with results obtained from manual and/or automated methods for red cell, white cell and platelet parameters. Morphological Terminology with reference to origin and correct application was also discussed. The lineage of all blood cells was followed through systematically from the common stem cell through all stages of development. A comprehensive account of both normal haematology and pathological haematology was given over the 4 week teaching programme. The course was designed to give trainees confidence in the preparation, staining and examination of blood films, be able to differentiate the white cell count into both normal and abnormal populations and finally recognise and comment on with confidence, abnormal film findings in an extensive range of common blood cell disorders.

The six students who attended this course were as follows: Angella Manele and Michael Taloifaga from the Solomon Islands; Josephine Leebrug from Yap, Federated States of Micronesia; Peia Tuakana from the Cook Islands; and Justina Gaviga, Noella Tasavi and Jonas Robson from Vanuatu.

Haematology 2013 students and PPTC staff

External quality assessment 2013

The PPTC in April of this year provided, in addition to its existing courses, a newly introduced External Quality Assessment course here at the Centre in Wellington from the 22nd April to the 10th May (3 weeks in duration).

CWM Hospital Laboratory in Suva, Fiji is to act as a reference laboratory for a national EQA programme that will provide its sub-divisional laboratories with an opportunity of participating in EQA which currently they do not have access to. The PPTC EQA Course which focused on the fundamentals of setting up an EQA programme proved to be very successful and covered the following topics:

- EQA as an important component of LQMS
- Sample selection for EQA programmes in specific disciplines
- Processing equipment and consumables
- Processing of samples, packaging and dispatch (haematology, transfusion medicine, biochemistry, microbiology/ serology)
- IATA regulations
- Selection of EQA co-ordinators/referees responsible for individual disciplines
- Result analysis and interpretative reporting for each discipline (haematology, transfusion medicine, biochemistry, microbiology/serology,
- Result collation and participation rates
- Laboratory management of an EQA programme
- Provider management of an EQA programme
- A practical EQA workshop for each discipline
- Monitoring and evaluation

Three senior staff from CWM hospital, Fiji, were nominated to attend and included Dr Litia Tudravu, Ravendra Prasad and Sunjit Parkash. The PPTC wishes to sincerely thank, Clare Murphy, Marita Smit, Dan Gyles, Russell Cole, Navin Karan, Nicky Beamish, Russell Hay, Richard Ward, Christine Story and Phil Wakem for their valuable contribution towards the delivery of this course.

Students and PPTC staff - EQA course, April 2013

Laboratory quality management 2013
Throughout May of this year the PPTC once again provided an intensive laboratory quality management course covering the practical implementation of quality manuals, documentation control and structure, standard operating procedures, and reporting and result processing. Emphasis was placed on "hands-on" experience in creating policies and documents that were relevant and adopted easily into Pacific Island laboratories. An extensive tour of Wellington, Hutt Valley Hospital laboratories and the NZ Blood service in Wellington were excellent in providing valuable experience and exposure to the daily working operations of accredited laboratories. To see these laboratories actively functioning and monitoring their performances under the confines of ISO15189 was very impressionable to our students, filling them with immeasurable enthusiasm and motivation to make it happen in their home Laboratories.

Students attending the course included Timoci Racolo from Labasa, Fiji; Theresa Tatuava from the Cook Islands; Cecil Tofirima from the Solomon Islands and Telesia Apikotoa from Tonga.

Country visits

The STI Programme:
The STI teaching programme (an initiative of WHO), has now reached completion and has provided laboratory technicians working in microbiology and serology sections of Pacific Island country laboratories with the information and skills needed to accurately perform the various laboratory tests related to the laboratory diagnosis of the common sexually transmitted infections [STIs] including HIV and also Hepatitis B & C.

This STI Programme was developed by the PPTC as a hybrid course and consisted of two main parts; a comprehensive theory component which was made available to Pacific Island students through distance learning covering all the STIs and RTIs [reproductive tract infections], and practical workshops that were conducted in National laboratories of participating countries (11 in total). This programme has been enormously successful and congratulations must go to both Russell Cole, our PPTC laboratory quality co-ordinator and Navin Karan our PPTC teaching and training programme co-ordinator for their contribution towards the successful delivery of this programme. Since October 2012, Russell and Navin have shared the responsibility for the delivery of STI workshops to the Cooks, American Samoa, Samoa, Marshall Islands, Yap, Vanuatu, Fiji, the Solomon Islands, Pohnpei , Kiribati and Tonga.

Marshall Islands haematology training
Phil Wakem visited Majuro (Marshall Islands) in May of this year to conduct an intensive two week haematology and blood cell morphology teaching programme for the laboratory staff of Majuro Hospital, and over a weekend was treated to a Marshallese picnic provided by the staff for which he was most grateful. The haematology course was very successful, and the students learnt a great deal from him. Russell Cole is scheduled to visit the Marshall Islands in November to conduct microbiology teaching and training.

Phil is also scheduled to visit the Rarotonga Hospital Laboratory (Cook Islands) in August to conduct a haematology teaching programme as well as an evaluation and assessment of the laboratory's EQA programme. He is also scheduled to visit the World Health Organization office in Manila towards the end of August and on the same trip carry out an evaluation of the EQA programme in Koror Hospital laboratory (Palau) situated very close to the Philippines.

LQMS visits
Both Navin and Russell are scheduled to re-commence laboratory quality management implementation in Kiribati, Samoa, Tonga and Vanuatu between July and December of this year.

Marshall Islands haematology training

Wellington based courses scheduled for the remainder of 2013

Biochemistry	15 July – 9 August
Microbiology	2 September – 27 September
Phlebotomy	7 October – 25 October
Blood transfusion science	4 November – 29 November

Online distance learning courses

The PPTC is currently offering six POLHN modules leading to the Diploma in Medical Laboratory Science [PPTC] and students are currently nearing the completion of laboratory technology, the first of the six modules to be launched in the 2013 cycle.

The Diploma course will be delivered over a two year period to students who have registered and have been accepted for the programme. The cycle of the programme began in March 2013 and the scheduled dates for the remaining modules to be released in 2013 and 2014 are as follows:

2013 modules

Haematology	9 June 2013
Biochemistry	16 September 2013
Examination Part 1:	November 2013

2014 modules:

Microbiology:	3 March 2014
Transfusion science:	2 June 2014
Laboratory quality management systems:	15 September 2014
Examination Part 2:	November 2014

Welcome to our new staff members
The PPTC Board of Management would like to welcome Lisa Muollo as its newly appointed financial co-ordinator. Lisa is a chartered accountant and carries with her a wealth of accounting experience from small businesses to large corporate companies. The PPTC would also like to welcome Linda Billington as it's newly appointed office administrative co-ordinator. Linda carries with her a wealth of experience in office administration. The PPTC is very fortunate to have both Lisa and Linda as part of its dynamic team .

Christine Story's retirement

Christine Story retired from the PPTC, on Friday 17th May 2013 after 30 yrs of devoted service to the institution. Christine has given to each and every one of us her generous support and guidance over the years, and the PPTC will miss her greatly for the enormous contribution she has made to the success of the PPTC as a teaching and training centre.

Her warmth, kindness and care towards all students that the PPTC has taken responsibility for has always been her strength and because of this, she belongs in a very special place among each and every one of them. We will miss her very much but wish her all the happiness and best wishes for the future.

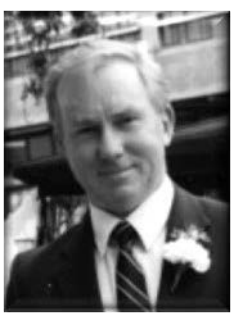
NZIMLS
Barrie Edwards & Rod Kennedy Scholarship

The Barrie Edwards & Rod Kennedy scholarship is one of the most significant awards offered by the NZIMLS. The scholarship provides the winner with support to attend an international or national scientific meeting up to a maximum value of $7,500.

Barrie Edwards

Application for this prestigious scholarship is invited from Fellows, Members and Associate Members of the NZIMLS. Applicants must be a current financial member of the NZIMLS and have been a financial member for at least two concurrent years prior to application. To be eligible applicants must make an oral presentation or present a poster as 1st author at their nominated scientific meeting.

All applications will be considered by a panel consisting of the President and Vice-President of the NZIMLS and the Editor of the New Zealand Journal of Medical Laboratory Science (who are ineligible to apply for the scholarships). The applications will be judged on your professional and academic abilities together with your participation in the profession. The panel's decision is final and no correspondence will be entered into.



Rod Kennedy

Application is by letter. Please address all correspondence to:

NZIMLS Executive Officer,
PO Box 505,
Rangiora 7440

There is one scholarship awarded in each calendar year. Closing date is December 20th in any given year. In your application letter please provide the following details:

- Full name, position, work address, email address and contact phone number
- The length of time you have been a financial member of the NZIMLS
- The conference you wish to attend – please provide dates
- A budget comprising airfares, conference registration and accommodation costs
- The abstract of your intended oral or poster presentation and whether it has been accepted for presentation (proof required)
- Your intentions to publish your results
- State briefly your history of participation in the profession over the last 5 years

State the reasons why you wish to attend your nominated scientific meeting. Successful applicants will be required to provide a full written report on return which will be published in the Journal. If not intended to publish elsewhere, successful applicants will be required to submit their study results for consideration by the New Zealand Journal of Medical Laboratory Science.

Journal questionnaire

Below are 10 questions based on articles from the August 2013 Journal issue. Read the articles carefully as most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using Microsoft's Internet Explorer.

You are reminded that to claim valid CPD points for successfully completing the journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. **In addition, members who have successfully completed the journal questionnaire can only claim 5 CPD points. You cannot then claim additional CPD points for reading the articles from which the questions were derived.**

The site will remain open until Friday 25th October 2013. You must get a minimum of 8 questions right to obtain 5 CPD points.

The Editor sets the questions but the CPD Co-ordinator Jillian Broadbent marks the journal questionnaire. Please direct any queries to her at cpd@nzimls.org.nz.

August 2013 journal questions

1. List four of the most common pitfalls encountered in the daily practice of haematopathology.

2. For bone marrow aspirate and trephine biopsy the preferred anatomic site is the posterior iliac crest. What alternative anatomic site may be sampled and under which conditions?

3. Why do bone marrow haematogones often cause diagnostic problems?

4. How have isotype controls in flow cytometry historically been used?

5. Patients with chronic granulomatous disease are especially susceptible to infections caused by which organisms?

6. What is the main characteristic of chronic granulomatous disease and what does it result in?

7. What can *Streptococcus agalactiae* infection cause in neonates and how are they infected?

8. Normal vaginal flora includes which organisms?

9. Eosinophilia is a phenomenon commonly associated with which conditions?

10. Which particular cytokines are suggested to be involved in the development of blood hypereosinophilia in cancer patients and what important role do these cytokines play in?

Questions and answers for the April 2013 journal questionnaire

1. How has *Neisseria gonorrhoeae* detection traditionally been performed?
   By growing and identifying viable organisms by microbial culture before measuring antibiotic susceptibilities.

2. Muco-purulent discharge, commonly associated with *N. gonorrhoeae* infection, can block the pipettor on the cobas 4800 analyser. How can this be overcome?
   By a 1:1 dilution of cobas collection buffer with Sputasol (1.4% DTT, Oxoid).

3. What are the advantages and disadvantage of using the BBL CHROMagar II MRSA media?
   Advantages are short hands on time, minimal personnel time required for reading cultures, and the low cost of the test in comparison to PCR methods. The disadvantage with using this media is the long turnaround times.

4. What does the LightCycler (LC) MRSA advanced test target?
   The test targets the integration site of the SCC*mec* cassette into the *S. aureus* chromosome with melting point analysis of the PCR product.

5. Why is high sensitivity of any MRSA test necessary?
   Because the objective of a MRSA screening program is to swiftly identify all those colonised with this bacterium, even if detection results from identifying DNA no longer associated with viable organisms.

6. If undiagnosed or untreated, *Clostridium difficile* infection can lead to what, and which antibiotics are commonly used to treat *Clostridium difficile* infection?
   Pseudomembranous colitis, toxic megacolon and death may result. *Clostridium difficile* infection is treated with metronidazole and/or vancomycin.

7. The failure to isolate *Clostridium difficile* from toxin A/B positive samples can be attributed to a number of factors. Name these.
   Suboptimal faecal storage conditions prior to culture, samples in which the organisms were no longer viable but toxin remained detectable, or cross reactivity of *C. sordellii* toxins with *C. difficile* toxins A/B.

8. All *Clostridium difficile* isolates were susceptible or resistant to which antibiotics?
   Susceptible to vancomycin, metronidazole, amoxicillin/clavulanic acid and meropenem. Resistant to ciprofloxacin.

9. Traditionally, what sweat chloride levels are regarded as supportive of cystic fibrosis diagnosis, what levels are deemed intermediate, and what levels makes cystic fibrosis diagnosis unlikely?
   Supportive: >60mmol/L. Intermediate: 40-60mmol/L. Unlikely: <40 mmol/L.

10. What does a CFTR gene defect in sweat glands result in?
    Reduced transport of sodium and chloride in the reabsorptive duct and therefore sweat with a higher salt content.

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NZIMLS Annual Scientific Meeting 2013

Bridging Science and Education

WORKSHOP PROGRAMME

- **Haematology / Morphology** with Sue Webber and Dr Helen Moore
- **Malaria** with Robyn Wells, RCPA QAP Malaria Programme
- **Pre-Analytical Effects on Tests of Haemostasis** with Dr Steve Kitchen, Scientific Director, UK NEQAS Blood Coagulation, Royal Hallamshire Hospital
- **Capture Technology in Blood Banks** with Graham Smallridge from Europe
- **Using Statistical Insights to Advance Laboratory Quality** with John C Yundt-Pacheco, Scientific Fellow and Dr Curtis Parvin PhD, Biostatistician, USA

Places still available in these workshops – don't miss out – register now!

Dr David Sacks
David Sacks is a Senior Investigator and Chief of Clinical Chemistry at the National Institutes of Health, USA.

Dr. Sacks obtained his M.B., Ch.B. from the University of Cape Town, South Africa. Further training included residencies in Internal Medicine at hospitals affiliated with Georgetown Medical School and in Clinical Pathology at Washington University School of Medicine. He spent 21 years at Harvard Medical School.

Dr. Sacks is Chair of the National Glycohemoglobin Standardization Program (NGSP) steering committee and a member of the International Federation for Clinical Chemistry Integrated Project for HbA1c. He has published over 150 peer-reviewed articles in scientific journals.

Steve Ktichen
Steve is currently the Scientific Director of the UK NEQAS for Blood Coagulation with an additional scientific role in the WHO and World Federation of Haemophilia EQA programmes. He is the head scientist at the Sheffield Haemostasis and Thrombosis Centre. He has 25 years experience in the field of laboratory testing in haemostasis with a long standing interest in standardisation and is active within the Science and Standardisation Committee of the ISTH. He has been involved in drawing up guidelines for a number of National and International bodies in the field.

Curtis Parvin
Bio-Rad Laboratories
Curtis Parvin joined Bio-Rad Laboratories in early 2009 where he serves as Manager of Advanced Statistical Research in Bio-Rad's Quality Systems Division. Prior to joining Bio-Rad, Dr Parvin spent almost 29 years on the faculty of Washington University School of Medicine as Director of Informatics and Statistics in the Division of Laboratory and Genomic Medicine. Dr Parvin's training is in biostatistics and medical informatics. A significant amount of his academic work involved the application of statistical theory to problems relevant to laboratory medicine. He has co-authored numerous papers that have appeared in several of the leading laboratory medicine journals. One of his primary research interests focuses on statistical methods for modeling and characterizing the performance of laboratory quality control strategies. Recent work centers on designing and implementing QC strategies that effectively manage the risk of reporting incorrect patient results. At Bio-Rad Laboratories, Dr Parvin continues his efforts to advance the state-of-the-art in laboratory quality control theory and practice.

Ellen Jo Baron
Professor Emerita, Pathology,
Stanford University Director of Medical Affairs, Cepheid, USA
Ellen Jo Baron is Professor Emerita of Pathology at Stanford University. She was the Director of the Clinical Microbiology & Virology Laboratory there starting in 1997, serving both the Stanford Hospital and the Lucile Packard Childrens' Hospital. From 2009 to 2013 she was Director of Medical Affairs for Cepheid, a molecular diagnostics manufacturer; now she is Exec. Director of Technical Support. She was the 2008 Chair of the Clinical Microbiology Division of the American Society for Microbiology (ASM). The Clinical Microbiology Task Force was started during her tenure as Chair. She is currently Divisional Group 1 Representative, planning the Clinical Microbiology & Epidemiology portions of the ASM General Meeting.

Amanda Zatta
Amanda is a NHMRC Peter Doherty Research Fellow in the Transfusion Research Unit, operating within the Critical Care Division at the Department of Epidemiology and Preventive Medicine, Monash University. Amanda was the Project Manager of the Australian and New Zealand Haemostasis Registry which collected 10 years of data on the administration of activated recombinant factor VII for treatment against critical bleeding in patients. More recently, Amanda is establishing a Massive Transfusion Registry (MTR) in Australia and New Zealand with the support of the Transfusion Outcomes Research Collaborative, which is a partnership between the Australia Red Cross Blood Service and Monash University and an educational grant from CSL Biotherapies and more recently, support from the New Zealand Blood Service. The aim of the registry is to collect data on the incidence, clinical contexts and outcomes of critical bleeding events that require a massive transfusion.

Robyn Wells
RCPA QAP
After completing her studies and traineeship, Robyn worked in the three major laboratories in Tasmania.

Robyn's interest in malaria and haematology morphology was further heightened by six months working in Saudi Arabia, where red cell disorders in particular, were prevalent.

After moving back to Brisbane in 1995, Robyn's expertise and morphological skills were enhanced by working in haematology at the RBWH and QUT. This was recognised by her promotion to Advanced Scientist in Haematology and the invitation to join the RCPA QAP Morphology Referees Committee. She was also instrumental in setting up the RCPA QAP malaria program.

2013 NZIMLS CALENDAR

Dates may be subject to change

Date	Event	Contact
20 - 23 August	NZIMLS Annual Scientific Meeting, Claudelands Event Centre, Hamilton ***Bridging Science & Education***	mary-ann.janssen@waikatodhb.health.nz
Date	**Seminars**	**Contact**
12 October	PreAnalytical SIG Seminar	tsheehan@dml.co.nz
19 October	Histology SIG Seminar, Nelson	alannah_z_@hotmail.com
23 November	Immunology SIG Seminar, Copthorne Hotel Commodore, Christchurch	angela.horridge@sclabs.co.nz
9 November	Mortuary SIG Seminar	bill.little@southerndhb.govt.nz
Date	**NZIMLS Examination Information**	**Contact**
6 November	QMLT and QSST Examinations	fran@nzimls.org.nz
12-13 November	Fellowship Examinations	fran@nzimls.org.nz
Date	**Council Matters**	**Contact**
3 August	Ballot papers to be with the membership (21 days prior to AGM)	fran@nzimls.org.nz
11 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM	fran@nzimls.org.nz
18 August	Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)	fran@nzimls.org.nz
18 & 19 August	Council Meeting, Hamilton	fran@nzimls.org.nz
22 August	Annual General Meeting, Hamilton	fran@nzimls.org.nz
Mid September	Material for the November issue of the Journal must be with the Editor	rob.siebers@otago.ac.nz
November	Council Meeting	fran@nzimls.org.nz

2014 NZIMLS CALENDER

Dates may be subject to change

Date	Seminars	Contact
March	South Island Seminar, Timaru	judy.dolman@medlabsouth.co.nz
23-25 May	NICE Weekend, Wairakei Resort, Taupo	raewyn.cameron@lsr.net.nz grace_agustin@nzblood.co.nz
Date	**Event**	**Contact**
12-15 August	NZIMLS Annual Scientific Meeting, Dunedin	terry.taylor@sclabs.co.nz

2015 NZIMLS CALENDER

Dates may be subject to change

Date	Event	Contact
18-21 August	NZIMLS / AIMS South Pacific Congress The Langham, Auckland	rossh@adhb.govt.nz