

APPENDIX 2.3.

INSTRUCTION MANUAL

IMPORTANT

DO NOT START SAMPLING BEFORE YOU HAVE READ AND UNDERSTOOD THE FOLLOWING INSTRUCTIONS.

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1. IMPORTANCE OF STUDY

Marginal vitamin A status is commonly associated with infectious diseases (respiratory infections, diarrhoeal disease and severe measles), immune dysfunction and increased mortality in children, in addition, it is associated with poor growth, iron deficiency anaemia and poor vision. The precise mechanisms by which vitamin A exerts its effects (excluding its role in vision) are not fully understood. Vitamin A is important in maintaining the integrity of epithelial tissues and immune function and a deficiency results in a loss of the first line of host defence barriers, predisposing the host to infections. Furthermore, iron deficiency is known to be associated with impaired growth and mental development. Vitamin A status and iron metabolism are known to be interrelated. Current evidence suggests that iron deficiency anaemia can be a consequence of inadequate intake of vitamin A and that vitamin A therapy improves iron status. The reason for the anaemia following vitamin A deficiency is unclear, but it is speculated that vitamin A is involved in the regulation of iron release from the liver.

This study is a combined effort of the The South African Vitamin A Consultative Group and the Department of National Health and Population Development to determine the vitamin A and iron status as well as immunisation coverage in children 6-71 months of age. Regional coordinators have been appointed and they in turn have appointed team leaders and field workers to help with the execution of the study. In order to ensure accurate and reliable data, the team leaders will supervise the data collection process and cross-check some of the information that you as field workers have obtained. If differences are found, you will be asked to repeat your observations in the presence of the team leaders in order to find the reasons for the differences.

It is also important that the questions be asked in a similar way by all the field workers and if you need to translate the questions, to ensure that the meaning of the question is not changed by the way that you rephrase the question.

The team leaders as well as the regional coordinator will be available for assistance and help at all times. Please ensure that you are familiar with their respective telephone numbers. Please feel free to contact them should you encounter any difficulties.

2. EQUIPMENT PER TEAM

BEFORE YOU LEAVE FOR THE SURVEY POINT ENSURE THAT YOU HAVE THE FOLLOWING EQUIPMENT WITH YOU.

- **Survey box, containing Cooler box**
- **Check list forms**
- **List of selected households**
- **Appropriate aliquots of vitamin A2**
- **Letters of introduction**
- **Consent forms**
- **Questionnaires**
- **Scale**
- **2 x Measuring boards (paediatric and adult)**
- **Map of the area**
- **Peanut butter and bread**
- **Torch**
- **Clip boards**
- **Antiseptic**
- **Sweets/or other incentives**

3. INSTRUCTIONS TO ALL REGIONAL COORDINATORS AND TEAM LEADERS

BEFORE THE SURVEY IS TO START

1. Prepare and pack the Survey boxes, according to the list under the section "Contents of the Survey box".
2. Prepare and pack the Accessories boxes as itemised on the Centre Point Accessories box list in the Instruction Manual.
3. Deliver the Centre Point Accessories box (the Centre Point being the Laboratories to be used in the various areas as arranged) to the appropriate Laboratories. Also include the "Instructions for Centre Points".
4. Confirm the arrangements regarding the transport of the blood samples with the Laboratories involved the day before the survey is to start.
5. Place three ice packs in a freezer or the freezer compartment of a fridge overnight.

Before despatching boxes to sampling stations the following morning, all regional COORDINATORS and team leaders MUST:

1. Ensure that three ice packs (now frozen from overnight) are placed in each of the cooler boxes.
2. Ensure that the Cluster Numbers and selected household addresses have been inserted on the Household Questionnaires.
3. Ensure that you have all the equipment indicated on Page 303 of this manual with you before departing to sampling points.
4. Ensure that the name of the contact person (and telephone number) at the Centre Point has been inserted in paragraph 15 of INSTRUCTIONS FOR SAMPLING.
5. Ensure that the CHECK LIST forms, INSTRUCTION MANUAL, CONSENT FORMS and QUESTIONNAIRES are located in the survey box.

On receipt of survey boxes from sampling stations, check with the Centre Point contact person that the arrangements for the transport of the cooler boxES containing the serum

and plasma samples, from the Centre Point to the analytical laboratory at Tygerberg Hospital in Cape Town, have been made.

Contact telephone numbers at the analytical laboratory at Tygerberg Hospital: During working hours TELEPHONE: Dr Moodie or Miss Louw (021) 938 5272 or Professor Labadarios or Mrs Blaauw (021) 938 9259; or fax (021) 933 2991. After hours TELEPHONE: Dr Moodie (021) 762 4710; Miss Louw (021) 94 2163 or Mrs Blaauw (021) 913 2627; Prof Labadarios (021) 96 8839.

When the survey, in all the areas in your Province, is complete, consolidate all unused items (including those at the Laboratories) and return them to Roche.

4. CONTENTS OF SURVEY BOX

- 1 x "INSTRUCTION MANUAL"**
- 1 x CHECK LIST FORM (Located in transparent plastic sachet.)**
- 60 x HOUSEHOLD QUESTIONNAIRES**
- 50 x INDIVIDUAL CHILD QUESTIONNAIRES**
- 25 x Consent forms for blood sampling**
- 2 x Consent forms for vitamin A2 analogue administration**
- 40 preprinted labels**
- 1 x Tourniquet**
- 8 x Pairs of gloves (medium size)**
- 20 x 5ml EDTA tubes (purple rubber top) contained in polystyrene trays as supplied by manufacturers.**
- 20 x 5ml Plain tubes (red rubber top) contained in polystyrene trays as supplied by manufacturers.**
- 20 x 5ml Disposable syringes**
- 16 x syringe needles**
- 16 x green Butterflies (21G)**
- 16 x vacutainers**
- 20 x Webcol sterile preps**
- 1 x Scissors**
- 3 x forehead thermometer strips**
- 16 x elastoplasts**
- 1 x pack of cotton wool**
- 1 x Black plastic sheet (50 x 50 cm)**
- 2 x Roller towel (paper)**
- 1 x Brown box to serve as waste disposal container (with sealing tape)**
- 1 x Polystyrene cooler box (with 3 ice packs)**
- 3 x Black pens (ball point)**
- 100 x paperclips**
- 1 x vitamin A2 analogue syringe**
- 1 x wet ones**
- 3 x clip boards**
- 3 x torches**
- 3 x bottles of hibitane**

5. CONTENTS OF EACH CENTRE POINT ACCESSORIES BOX

35 x Wasserman tubes (pale blue caps)

40 preprinted labels

1 x Roll of parcel sealing celotape

Labels with address of the Analytical Laboratory in Tygerberg Hospital

6. INSTRUCTIONS TO FIELD WORKERS

Field workers

A team consists of at least 3 people.

This team must:

1. Visit the selected households indicated on the household listing for the cluster.
2. Explain the purpose of the visit.
3. Find out whether any children from 6-71 months of age live in that household.
4. Fill in a household questionnaire for the household.
5. Fill in an individual child questionnaire for each child aged 6-71 months.
6. Measure the height and weight of each child aged 6 - 71 months.
7. Check each child's eyes for signs of vitamin A deficiency and neck for visible goitre.
8. Determine the temperature of each child that is hot to touch.
9. Give the first child where permission was obtained for a blood sample, vitamin A2 analogue. This child must be visited again after 4-6 hours and then the 5 ml of blood taken.
10. Take 5 ml blood from 16 children in each cluster where a parent has given permission.

Deliver questionnaires and blood samples to designated areas (i.e. Centre Points) at designated times. This will be arranged by the coordinators and will differ for each region.

6.1 Visit the SELECTED households indicated on the household listing for the cluster

- Write the street address on the household questionnaire. This address must be the same as the address on the household listing.
- Indicate the date and time of the visit on the questionnaire.
- If there is no-one at home, this household must be visited a second time. Find out from the neighbours when they are usually at home and if there are any children.
- If at the second visit no-one is at home, indicate it on the household questionnaire and consider it as complete.

- Only the households indicated on the listing should be visited.
- No substitution must be done.

6.2 Explain the purpose of the visit

- One of the following people may be interviewed :
 - The mother or father of the child.
 - The caretaker of the child if older than 16 years of age (The caretaker may be a grandparent, older sibling, relative or other person).
- Introduce yourself and ask to speak to the person in charge of the household. Give them the letter of introduction.
- The purpose of the visit is to get an idea of the growth, vaccination status and vitamin A status (an important vitamin to help you see and fight infections) of preschool children all over the country.
- This will enable us to help the government start programmes throughout the country to improve children's health.
- To be able to do this, we must ask you some questions about your family and your house, look at your child/children's "Road to Health chart", measure your child/children's height and weight and if you are in the lucky first 16 of this area, take 5 ml of blood from your child/children.
- If we find any abnormalities in your child/children, we will give you a referral letter to take to the nearest clinic for the appropriate treatment.

6.3 Find out whether any children AGED 6-71 months live in that household.

- All the children in this age-group that live in that household (not visitors, daycare kids etc) must be included. Fill their full names and surnames in on the household questionnaire next to the numbers 1 - 7, where "1" indicates the youngest and "7" the oldest child.
- If there are no such children living in that household, indicate it on the household questionnaire and consider it as completed.
- No substitution must be done.
- If more than one family live in the household or on the property, they must be included.
- All the children in the given age group must be included. If they are not all at home, arrange to come back.

6.4 Fill in a household questionnaire for the household

- Complete the rest of the household questionnaire.
- One household questionnaire per family must be completed.
- Insert the Family number in the block next to the Household Number, starting from 1.
- "Most of the week" means more than 4 of the 7 days of the week.

6.5 Fill in an individual child questionnaire for each child from 6-71 months.

- A separate form must be completed for each child aged 6 - 71 months. Mark the forms clearly by inserting the number 1 - 7 that appears next to the child's name on the household questionnaire in the study number.
- Name of interviewer :
 - Please insert your name and team number in the space provided.
- The immunisation card/Road to Health card of each child must be examined, if possible. The parent or caretaker of the child or another child, if older than 16 years of age, can get this card for you.
- **NB.** Write the study number of the child on the Road to Health card in the top left hand corner.
- If there is no card, the parent or caretaker of the child can give the immunization information from memory.

• Measurement methods :

1. Date of birth : In order of preference:
2. Date of birth (documented - Road to Health card / birth certificate).
3. Date of birth (maternal recall).
4. Year of birth (maternal recall).
5. Or age :
6. Age (maternal recall).

• Sex :

1. By observation.
2. By questioning caretaker.

• Immunization :

1. By copying "Road to Health" card.

2. By history (maternal recall).

- The immunization source key "P" for Private, includes all sources of private immunization eg. private clinics, pharmacists etc.
- If the child is older than one year and all the shaded blocks of the vaccination status table have not been filled in with either a date or a "+", ask the following question on the questionnaire in a friendly, non threatening manner and write down exactly what the mother/caretaker says.
- If the answer to this section is the only outstanding information, you need not return to that household.

● **How long breastfed:**

1. Maternal recall.
 2. Other caretaker recall.
- If the child was breastfed for less than one month, it should be indicated as no breastfeeding.

● **Clinical examination :**

- This should be done according to the instructions under section 6.6 of this manual.

● **Anthropometry :**

- Weight and height must be measured according to the instructions under section 6.7 of this manual.

● **Medical history :**

- This section should only be completed for those 16 children from whom a blood sample is taken.
- Fill in the rest of the individual child questionnaire by completing the questions. If the child is hot, determine the temperature according to the instructions under section 6.8 of this manual. If in doubt, also determine the temperature.
- The definition for diarrhoea for this study, is a change in the normal stool pattern and more than 3 stools per day.

● **Blood sampling :**

- Follow the instructions under sections 6.9 and 6.11 of this manual.

● **IMPORTANT :**

Before leaving a household check that :

- you have a completed household questionnaire for every family in that household.

- you have individual child questionnaires completed for each child between the ages of 6-71 months in that household.
- you check the accuracy and completeness of the information on the questionnaires and sign in the appropriate block at the top of the Individual Child Questionnaire to indicate that you have checked the information.

6.6 Instructions for clinical examination

• Eye examinations :

- Check each child's eyes for signs of vitamin A deficiency. Compare with the eye chart and indicate your finding in the appropriate block.
- To distinguish between keratomalacia and blindness, ask whether the child has any light perception. If the answer is no, indicate it as blindness.
- Ask if the child has difficulty seeing / finding things in the dark.

• Visible goitre :

- Check each child's neck for visible goitre with the neck in a straight and extended position. Please mark as "unsure" if you are unsure.
- NB. If any abnormality is suspected, bring this to the attention of the team coordinator for a second opinion.

• BCG scar :

- By examination of child's right upper arm deltoid area (shoulder).

6.7 Instructions For Anthropometric Measurements

• Determination of weight :

1. The scale must be placed on an even uncarpeted area. Ensure that the spirit level indication is in the middle.
2. Switch the scale on and wait for the zero indication (0,0) as well as the stable indicator (o in the top left hand corner of the display panel) to appear.
3. Weigh the children (preferably after emptying their bladders) with minimum clothes on
 - diapers only for babies (dry only)
 - underclothes for older children
4. Place the child on the scale. They should be standing upright in the middle of the platform, facing the field worker and looking straight ahead. They should stand with their feet flat and slightly apart and should remain still until the reading has been taken.

5. Write the reading down in the space provided on the questionnaire before removing the child from the scale. The measurement should be recorded to the nearest 100g.
6. Have the child step down from the scale and wait for the zero reading to appear on the digital display.
7. Repeat procedure. The 2 readings should not vary by more than 100g. If they do, check the scale for accuracy and repeat the procedure until the correct weight is obtained.
8. If the child/baby is not able to stand alone on the scale
 - a. Follow procedures 1 and 2 as described above
 - b. Weigh the mother/caretaker first (without heavy clothing and shoes)
 - c. Press the zero/reset button and wait for the zero reading (0,0) to appear on the
9. digital display
10. Place the baby in the mother's arms. Write the reading down in the space provided on the questionnaire.
11. Remove mother and child from the scale and wait for the zero reading to appear.
12. Repeat the procedure
13. Ensure that the scale is plugged in overnight to recharge the batteries.
14. When the scale is not in use, it switches off automatically after a few minutes.

- **Determination of height :**

1. **Children younger than 2 years:**

1. Place the measuring board on an even uncarpeted area.
2. Ensure that the measuring board is functional and the footboard has not got undue loose movement.
3. Place the child lying on its back on the board with the crown of the head touching the fixed headboard. The shoulders should be touching the base of the board. One person is needed to hold the child in this position.
4. A second person ensures that the child's heels are touching and the legs are stretched (knees not bend), and slides the footboard against the soles of the child's heels and takes the measurement to the nearest mm.
5. Take the measurement on the inside of the footboard to the nearest 0,1 cm.
6. Write the measurement down in the space provided on the questionnaire and repeat. The 2 readings should not vary by more than 0,5 cm.

2. Children older than 2 years.

1. Place the stadiometer on an even uncarpeted area.
2. Remove the child's shoes.
3. Position the child as follows :
 - facing you
 - shoulders relaxed, with shoulder blades, buttocks and heels touching the measuring board
 - arms relaxed at sides
 - legs straight and knees together
 - feet flat, heels touching together
4. With child looking straight ahead (Frankfurt plane), slide the headpiece down until it touches the crown of the head.
5. Take the reading to the nearest mm.
6. Write the measurement down in the space provided on the questionnaire and repeat. The 2 readings should not vary by more than 0,5 cm.

6.8 Instructions For Taking Temperature

- If the child's forehead is hot to touch with the dorsal aspect of the investigator's hand, or if in doubt, use the thermometer strip to determine the child's temperature.

• How to use the forehead thermometer :

1. Remove the thermometer from the plastic box.
2. By holding the thermometer at each end, firmly press the thermometer flat against
3. The child's temperature will be indicated by whichever symbol turns green.
4. Consult the following chart to translate the symbols.

-	√	+	++	!
36°	37°	38°	39°	40°

5. Record the appropriate temperature in the space provided on the questionnaire.
6. Wipe the thermometer clean after use with a damp paper towel and replace in the plastic box.
7. Avoid prolonged exposure of the thermometer to direct sunlight.

6.9 Instructions for the administration of Vitamin A2 analogue

- Give the Vitamin A2 analogue to the first child between the age of 12-71 months in the first household where the parent has given permission for blood to be taken. This analogue should only be given to one child per cluster.
- If there is more than one child in the household, administer the vitamin A2 analogue to the child selected according to the selection table in section 6.10 of this manual.
- If permission is not given, then ask the parent of the next house until permission is received. Only one child per cluster needs to be given the vitamin A2 analogue.
- The following procedures should be adhered to :
 - Select the child to receive the vitamin A2 analogue by obtaining consent from the parents of the first selected child between the age of 12 - 71 months in the first selected household. Remember to use the correct analogue consent form.
 - If the parent is illiterate, a cross (X) can be made on the form with the signature of a witness next to it.
 - Explain the procedure to be followed to the parent.
 - Prepare a snack containing « - 1 slice of white bread and 20 grams of peanut butter.
 - Carefully suck up 180 æl of the vitamin A2 analogue by using the syringe provided. This is done by firstly ensuring that the plunger is pushed all the way down. Then insert the tip of the syringe into the analogue (to a depth of 1-2 mm), and carefully draw up the fluid until the bottom of the black plunger reaches the 0.2 ml mark. Ensure that there are no air bubbles in the syringe.
 - Administer the analogue onto the peanut butter by slowly pushing the plunger all the way down.
 - Ensure that the child consumes all the peanut butter as this is essential for the absorption of the vitamin A2 analogue. If the child cannot eat bread, the peanut butter can be mixed with porridge, provided that everything is consumed and no milk is added.
 - For the next 5 hours, until the blood sample is taken, the child may not have any food containing vitamin A, e.g. no full cream dairy products. It is very important that the parent and child understand this.
 - Arrange with the parent for a time 4-5 hours later that you will return to draw blood from the child. Indicate this arranged time in the appropriate space on the consent form.
 - Remember to go back to the child at the arranged time to draw the blood.
 - Remember to indicate on the Individual Child Questionnaire which child received the vitamin A2 analogue by inserting an "A" in the last block of the study number.
 - Please keep the amber coloured vials after administration of the analogue and return them to the team leaders at the end of the day.

- o Remember to indicate at the bottom of the first page of the Individual Child Questionnaire that the child received vitamin A analogue.

6.10 Selection Of Child To Receive The Vitamin A2 Analogue

If the number of children 6-71 months in the household is:								If the number of children 6-71 months in the household is:							
1	2	3	4	5	6	7+		1	2	3	4	5	6	7+	
then, for A2, select the number:								then, for A2, select the number:							
WC01	1	2	1	1	3	1	4	WC21	1	1	2	1	2	5	5
WC02	1	1	2	2	1	6	1	WC22	1	2	3	1	3	4	3
WC03	1	2	2	1	5	5	5	WC23	1	2	1	1	1	4	1
WC04	1	1	2	3	1	2	1	WC24	1	2	3	3	4	3	5
WC05	1	2	3	1	2	3	1	WC25	1	1	1	4	5	6	4
WC06	1	2	3	4	4	3	1	WC26	1	2	3	1	4	1	5
WC07	1	2	2	4	1	5	1	WC27	1	2	2	3	2	4	5
WC08	1	2	3	2	2	3	4	WC28	1	2	3	4	3	1	4
WC09	1	2	3	2	2	5	2	WC29	1	1	1	4	3	5	1
WC10	1	2	3	4	2	1	1	WC30	1	2	1	1	5	4	2
WC11	1	1	2	3	1	2	7	WC31	1	1	1	1	5	6	5
WC12	1	2	3	3	2	2	1	WC32	1	2	1	3	4	2	6
WC13	1	1	2	4	2	1	1	WC33	1	2	2	2	2	3	7
WC14	1	2	2	4	1	5	3	WC34	1	1	3	2	3	5	4
WC15	1	2	1	1	4	6	1	WC35	1	2	3	4	5	6	7
WC16	1	1	2	1	2	1	2	WC36	1	1	3	3	3	1	4
WC17	1	2	2	4	5	3	2	WC37	1	2	2	2	1	3	7
WC18	1	1	1	4	3	6	5	WC38	1	1	1	1	2	3	7
WC19	1	1	3	3	4	5	3	WC39	1	2	2	3	2	6	7
WC20	1	1	2	4	5	2	5	WC40	1	2	3	3	3	1	1

6.11 Instructions for blood sampling

Take 5 ml blood from the next 15 children in each cluster where a parent/legal guardian has given permission. It is likely that it will take more than the first 15 households, but do not despair. Remember that you need a full 5 ml of blood from 15 children.

If there are blood from 14 children already and the next household has more than one eligible child, take blood from all.

NB. Obtain consent before proceeding. If they refuse, thank them for their cooperation and move on.

- If the parent is illiterate, a cross (X) can be made on the form with the signature of a witness next to it.
- If neither the parents/legal guardian is home, blood may not be taken. One can either come back a second time when they will be home or take blood from the next household selected for the study. Blood needs to be taken from 16 children per cluster (1 after the administration of the analogue and a further 15 where no analogue was administered).
- The only exclusion criterion is (1) if the parent has not given consent, (2) if the child's temperature is 38°C or more on the day of the survey or (3) if the child has received high-dose vitamin A supplementation (i.e. more than 100 000 IU) in the last six months.
- Remember to indicate at the bottom of the first page of the Individual Child Questionnaire that the child received vitamin A analogue.

CHECK THAT THE SIGNED CONSENT FORMS HAVE BEEN OBTAINED BEFORE PROCEEDING WITH SAMPLING.

1. Write CLEARLY on two labels (supplied) the study number, patient's name, surname, and date of sampling using BLOCK CAPITAL LETTERS.
2. Retrieve sample tubes from cooler box, replace lid and keep cooler box closed at all times.
3. For each patient, select one tube with purple rubber top and one tube with red rubber top. Stick labels (one on each tube) directly over label already on the tube.

4. Slowly and carefully draw a 5ml sample of venous blood (without stasis) into the syringe using a butterfly (needles are provided for those personnel who prefer to use them).
5. Write the time of sampling on the tube labels.
6. Slowly and carefully transfer from the syringe 1 ml of the sampled blood into the purple rubber top tube and the remaining blood into the red rubber top tube by piercing the rubber seals with the butterfly needle. NB : Should it be impossible to draw more than 1 ml of blood, ensure that all the blood is transferred into the purple rubber top tube. The vacuum within the tube will tend to draw the blood into the tube. Be ready to control this flow and direct it onto the inner wall of the tube NOT directly at the bottom of the tube. THIS IS IMPORTANT AS RAPID AND UNCONTROLLED FLOW WILL RESULT IN HAEMOLYSED BLOOD.
7. If less than 5ml of blood is obtained, ensure that 1ml of blood is carefully transferred into the purple rubber top tube and the remainder into the red rubber top tube. The bottom end of the label indicates the 1 ml mark.
8. Gently and repeatedly (5 times) invert both tubes. DO NOT SHAKE.
9. Place both tubes in polystyrene trays. KEEP TUBES SHIELDED FROM DIRECT LIGHT OR SUNLIGHT BY COVERING TRAYS WITH THE BLACK PLASTIC SHEET PROVIDED. Direct light is known to decompose vitamin A.
10. All blood samples drawn must be returned to the cooler box within two hours from the time the blood sample was drawn.
11. Dispose of syringe and butterfly (needle) in waste disposal container.
12. Proceed with the next patient (i.e. by repeating steps 2-9 as indicated above) until a complete blood sample (5ml) has been obtained from 16 patients.
13. Only two attempts should be made to draw blood from a particular child.
14. On completion of all sampling:
 - a. complete Section A of the Laboratory checklist
 - b. dispose of the gloves in the waste disposal container and seal it with the tape provided check that you have correctly filled in all details on the labels and that you have the correct number of samples.
 - c. close the cooler box and place it together with the sealed waste container and all other equipment supplied into the survey box.
15. Return survey box and contents to the predetermined Centre Point within 24 hours after the blood was drawn advising the Centre Point of estimated time of arrival.

NB: CONTACT PERSON AND TELEPHONE NUMBER AT YOUR CENTRE POINT:

- TELEPHONE :
- DR/MR/MRS/MISS :

THIS NUMBER IS ALSO TO BE CONTACTED IN CASE OF DELAY WHILST SAMPLES ARE IN TRANSIT TO THE CENTRE POINT.